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ON STOCHASTIC PROCESSES IN BIOLOGY

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The purposes of this article are: (i) to discuss the role of the theory of stochastic processes in the methodology of mathematical biology, (ii) to present a review of some work dealing with the application of stochastic processes in biology, and (iii) to encourage, perhaps, other workers to utilise the theory of stochastic processes in formulating mathematical models of various biological phenomena.

I. INTRODUCTION

Mathematical biology is concerned with the development of a *rationale* for biological phenomena. Before the researches of N. Rashevsky[2] (31) towards the development of a mathematical biology numerous studies were available in what might be termed mathematical ecology (Volterra, Kostitzin, Gause) and mathematical genetics (Fisher, Haldane, Wright). In the early days of mathematical biology the methods used by Rashevsky and his associates were mainly those of classical mathematical physics, with models of biological phenomena being postulated which could be treated by utilising these methods. It soon became evident, however, that the complex phenomena associated with the biological world could not be fully investigated by the mathematical methods of physics alone, and that new methods designed, perhaps, especially for biology would be required. That this situation might arise should come as no surprise when we reflect on the uniformity of physical and biological processes. In dealing with physical processes we have few variables between which to establish a functional relationship. Hence in physical experiments we need only to make a few determinations in order to predict the outcome of future experiments. This is an assumption on the uniformity of physical processes, and we find that it is deterministic. When we consider biological processes we find in the main a probabilistic or stochastic uniformity, a deterministic uniformity existing for only a few phenomena.

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[2]Numbers in parentheses indicate Reference cited.

With the above in mind it is perhaps clear why the formulation of abstract biological models should proceed more along stochastic rather than deterministic lines. Consider, for a moment, what we do in a biological experiment: We endeavour to measure the result of a series of interacting phenomena which vary in space as well as in time. We are unable, due to our ignorance of biological organisation, to establish an exact relationship between the interacting elements which we have been able to ascertain as playing a role in the reactions leading to the measured result. Hence any attempts to give an exact relationship between biological observables are usually futile, or lead to complex models of the underlying mechanism which defy experimental verification or refutation. In developing mathematical theories in biology we should postulate models which answer the following sort of question: If observations on a biological system are taken as elements of the set of all possible observations, what type of model will enable us to predict observations that as a rule will be elements of a specified subset of the set of all possible observations on the system, while it is possible that the observations will not be elements of the subset? At present the theory of probability and stochastic processes is the only mathematical scheme suitable for answering such questions, and hence formulating the desired mathematical theories in biology.

II. SOME REMARKS ON STOCHASTIC PROCESSES

In various problems treated by probabilistic methods one finds that the distribution of a random variable depends on a non-random variable which is a continuously varying parameter such as time. In these cases we speak of a *stochastic process*. Processes may be continuous or discontinuous depending on the distribution of the random variable. The theory of stochastic processes is now highly developed (9), (20); and there exists a large literature on the theory itself and its various applications. Outstanding applications are in fields such as physics, biology, and economics.

In this section we wish to make a few remarks about some of the processes that have been used in various biological applications. We will be concerned only with a brief characterisation of these processes, and defining some terms that will be used later.

1. *Markov chains.* Let x_0 , x_1 , x_2 , $\cdots$ be a sequence of random variables, each of which can take on one of the values ω_1 , ω_2 , $\cdots$. Let p_{ij} denote Prob $\{x_n = \omega_j \mid x_{n-1} = \omega_i\}$; that is p_{ij} is the probability that x_n has the value ω_j given that x_{n-1} had the value ω_i . The values ω_k are called the *states* of the system defined by the matrix of transition probabilities $P = (p_{ij})$. A matrix P is called *stochastic* if all of its

elements are non-negative, and the row sums are equal to unity. The matrix P together with the initial probability distribution $\{\alpha_k\}$ for the states ω_k at time zero completely defines a *simple Markov chain* (11), (13), (35). A chain is said to be *finite* or *infinite* depending on the number of possible states ω_k, and *discontinuous* (discrete) or *continuous* depending on whether transitions between states take place at integral values of time or not.

While the characterisation of a process as a simple Markov chain makes available for its study a well-developed theory, it imposes the restriction that the previous history of the system plays no role in determining its future course. In contradistinction to most physical systems, the future of biological systems is influenced by the past in a manner which leads one to believe that the past seems to reach out and grasp the future. Therefore in the study of biological phenomena as stochastic processes it is of interest to take these "hereditary" effects into consideration. This can be done by formulating the process as a *multiple Markov chain*. Multiple chains have transition probabilities depending on previous values of the random variable at more than one point, e.g., for a chain of order h,

$$p_{i-h+1,\cdots,i,j} = \text{Prob}\,\{x_n = \omega_j \mid x_{n-1} = \omega_i\,, \cdots\,, x_{n-h} = \omega_{i-h+1}\}.$$

The order of the process refers to the number of previous values of the random variable which are used in defining the transition probabilities. Available work on multiple chains is mainly concerned with the reduction to simple chains.

2. *Branching processes.* These processes represent the evolution or development of systems whose components can reproduce, be transformed, and die; the transitions being governed by probability laws (14). We can characterise these processes in the following manner: Consider a sequence of random variables $z_1\,, z_2\,, \cdots$. Let a single organism at time $t = 0$ have probabilities $p_0\,, p_1\,, p_2\,, \cdots\,, p_n$ of having n-offsprings. The variable z_n is interpreted as the number of organisms in the n-th generation. Let $p_{ij} = \text{Prob}\,\{z_n = j \mid z_{n-1} = i\}$ be the conditional probability that there are j organisms in the n-th generation given that there were i organisms in the $(n-1)$-st generation. We define a generating function for the probabilities p_k as follows

$$F(s) = \sum_{k=0}^{\infty} p_k s^k, \qquad |s| < 1, \qquad p_0 > 0;$$

then assuming that the organisms of a generation reproduce independently we have p_{ij} is equal to the coefficient of s^i in $[F(s)]^i$, and the process is a Markov chain determined by the matrix $P = (p_{ij})$. We

have also $p_{ij}^{(n)}$ is equal to the coefficient of s^i in $[F_n(s)]^j$, where $F_n(s) = F[F_{n-1}(s)]$; that is $F_n(s)$ is the n-th iterate of $F(s)$.

Since the publication of W. Feller's paper (10) on a stochastic model of population growth, the theory of branching processes has found many useful applications in biology. A discussion of this work is not included in this article since the recent papers of M. S. Bartlett (4), D. G. Kendall (18), W. Feller (12), and P. Armitage (2) give a review of earlier work, as well as new results in the study of genetics, population growth, and bacterial mutations. A recent application of a branching model is given in a paper by S. Iverson and N. Arley (15) on the mechanism of experimental carcinogenesis. In this theoretical and experimental study a branching model is postulated for the growth process in tumours, and the parameters occurring in the model are estimated from the experimental observations.

R. Bellman and T. E. Harris (7) have recently discussed a non-Markovian generalisation of the process presented above. In this generalised process, termed "age-dependent", the generation time τ from the birth of an organism until its own subdivision has the general distribution $dG(\tau)$, $0 < \tau < \infty$. The fundamental equation in the Bellman-Harris theory is the functional equation

$$F(z, t) = \int_0^t h[F(z, t - \tau)]\, dG(\tau) + z[1 - G(t)].$$

In the above equation $F(z,t)$ is the generating function for the number of organisms in the population at time t, and $h(z) = \sum_{n=0}^{\infty} q_n z^n$, where q_n is defined as the probability that an organism will give rise to n-offsprings when division takes place. In their paper only the case of binary fission was considered, i.e., $h(z) = z^2$. We have recently treated the case in which $h(z) = \sigma + (1 - \sigma)z^2$, i.e., the process considered is a modified birth-and-death process (33).

Before the appearance of the Bellman-Harris paper D. G. Kendall (17) had introduced the idea of a variable generation time in the study of stochastic birth processes. The case treated by Kendall is obtained by letting $G(t)$ be the k-fold convolution of the function $1 - e^{-\lambda t}$. This is equivalent to requiring the organism to pass through k stages before division can take place. The data of C. D. Kelly and O. Rahn (16) on the growth rate of *Bacterium aerogenes* was used to evaluate the parameter k, and to show the applicability of the model to biological problems.

The Bellman-Harris theory should have important biological applications (19), especially when other forms for $G(t)$ are considered, and the transformation probabilities q_n are made functions of time,

and the population size. It is also of interest to design experiments which will enable us to determine the value of the various parameters occurring in age-dependent process.

3. *Statistical inference in Markov chains.* In this section we wish to call attention to several studies concerned with statistical inference in Markov chains which should be of interest to biologists who want to test the adequacy of a postulated Markov chain model, or to see if their observations form a Markov chain.

T. W. Anderson (1) has considered a Markov chain model, with stationary transition probabilities, for analysing time changes in attitudes. The unspecified parameters in the model were the transition probabilities p_{ij} . Utilising experimental data the p_{ij}'s were estimated, and their statistical properties studied. Methods are also given for testing the hypothesis that: (i) the $p_{ij} = p_{ij}^{*}$ (a given number) for a specific i and j, (ii) the p_{ij} are not time dependent, and (iii) the process is of order one against the alternative that it is of order two. The results obtained are also generalised for multiple chains of order h.

Recently M. S. Bartlett (5), (6) has investigated the problem of testing the goodness of fit of a theoretical model to a Markov chain of order h. A method is also given for finding the maximum likelihood estimates for the transition probabilities of a chain of order h.

In two papers M. Ogawara (23), (24) has studied stationary processes of order h; and has given conditions, in the form of stochastic difference equations, that these processes and their autocorrelation coefficients must satisfy if they are to be of order h.

III. APPLICATIONS

1. *Central nervous system.* In 1948 A. Shimbel and A. Rapoport published a paper in which a probabilistic approach to the study of the structure and function of the central nervous system was developed (37). In this, and subsequent papers, a stochastic, rather than a deterministic, theory of neural nets has been developed and applied to numerous problems in neurobiophysics. When one considers that the human brain contains about 10^{11} neurons, which are the basic units of which it is built, it is perhaps clear why a stochastic approach is needed, and why it has proven to be a fruitful one.

In this approach the parameters which are associated with the neurons (threshold, refractory period, etc.) are assumed to vary from neuron to neuron according to some distribution function. The patterns resulting from the various neurons interacting with one another are characterised by certain functions of point paths in space. These functions denote the probability that a neuron in a macroscopically

small region about one of the points receives an axone from a neuron in a microscopically small region surrounding the second point. Hence the system is viewed as an aggregate of neurons characterised by continuous distributions of "local properties" together with certain tendencies of connection from region to region.

The probabilistic approach to the study of the central nervous system has given rise to the very useful concept of a random net (39). A random net can be defined as follows: Consider a collection of points, each of which issues some number of outwardly directed lines (termed axones). Each axone terminates upon some point of the collection, and the probability that an axone from one point terminates on another point is the same for every pair of points in the collection. The resulting configuration is a *random net.* A random net has two important properties which we shall now define. The *weak connectivity* of a random net is the expected number of points in the collection to which there exists a path from an arbitrary point, if the points are not in any way distinguished from each other. The *strong connectivity* is defined as the probability that from an arbitrary point in the net there exist paths to every other point. R. Solomonoff and A. Rapoport, using an approximation method, have computed the weak connectivity when the number of points in the net, N, is large, and when a, the number of axones issued by any point, is greater than or equal to one. When N and a are both small Markov chain methods are applicable to the study of randon net structure. Recently R. Solomonoff (38) has obtained exact expressions for the weak and strong connectivity utilising a multiple Markov chain of order 2.

Recent studies have been concerned with such topics as cycles and steady state phenomena in random nets, the spread of excitation in a random net, and nets with a distance bias (28), (29). In addition, A. Shimbel (36) has developed a learning theory, based on the lowering of thresholds of neurons, and applied it to several random net models.

2. *Radiobiology.* In the study of radiobiological phenomena several Markov chain models have been postulated. In 1945 I. Opatowski (25) developed a general theory of chain processes and applied his results to the study of injury and recovery in biological systems following irradiation. In Opatowski's theory the occurrence of an effective event in the sensitive volume of an organism is considered as a transition of that volume to a new state. The observed effect is reached after a certain number of transitions between states have taken place, so as to reach some final state which represents the observed effect.

The problem was formulated as follows: Consider a system which can take $n + 1$ states $(0, 1, 2, \cdots, n)$. Let the probability of transitions

$(i - 1 \to i)$ and $(i + 1 \to i)$ during any time dt be, respectively, $k_i dt + o(dt)$ and $g_i dt + o(dt)$. Let these be the only transitions possible during dt, and let the system be in the state 0 at t equal to zero. If $Y_i(t)$ is defined as the probability that the system be in the state i at time t, then $Y_0(0) = 1$, $Y_i(0) = 0$ for $i \geq 1$, and

$$\frac{dY_i}{dt} = k_i Y_{i-1} - k_{i+1} Y_i + g_i Y_{i+1} - g_{i-1} Y_i , \tag{2.1}$$

where $Y_{n+1} = Y_i = g = 0$ if $j < 0$. In the biophysical applications of (2.1) only the function $Y_n(t)$ and $k_i = k$ or 0, $g_i = g$ or 0, where k and g are positive constants, was considered. The k_i's depend on the intensity of the radiation and on the sensibility of the organism in the state $(i - 1)$ to a given radiation. Consequently, the k_i's depend on the probability of collision between a photon and the sensitive volume. The g_i's represent the intensity of recovery of the organism from the state $(i + 1)$ to the state i. By the method of the Laplace transformation an explicit expression for $Y_n(t)$ under the above assumptions was obtained.

The theoretical results obtained by Opatowski have been applied to the experimental work of A. Zuppinger (40) on the irradiation of *Ascaris* eggs with X-rays (26).

Recently A. T. Reid and H. G. Landau (34) postulated a model for the transmission of radiation damage following the initial damage due to the absorption of radiation quanta. The mechanism by which this transmission takes place was assumed to be the depolymerisation of macromolecules associated with the sensitive volume of the organism. The mathematical problem was formulated in terms of a random-walk problem with two absorbing barriers. A Markov chain with a finite number of states was considered: $E_0 \to E_1 \to \cdots \to E_{a-1} \to E_a$. The system was considered in the state E_0 initially, and following a "hit" in the sensitive volume passes to the state E_1 . The transitions, $E_1 \to E_2 \to \cdots \to E_a$, represent the transmission or amplification of the initial damage, and E_a represents the state when the radiation damage becomes observable.

The transition probabilities are defined as follows:

$$p_{i,i+1} = j/a, \qquad (j = 1, \cdots, a - 1), \tag{2.2}$$

$$p_{i,i-1} = 1 - j/a, \qquad (j = 1, \cdots, a - 1), \tag{2.3}$$

$$p_{ii} = \begin{cases} 1, & j = 0, a \\ 0, & \text{otherwise.} \end{cases} \tag{2.4}$$

The forward transition probabilities (2.2) represent transmission, and the reverse transition probabilities (2.3) represent recovery. The condition (2.4) means that no change will occur after either the state E_0 or E_a has been reached, or in the terminology of random-walk there are absorbing barriers at 0 and a. It also means that the system will not remain in any of the intermediate or interior states indefinitely, but will pass to either E_0 or E_a .

It was of interest to determine the probability, λ_a (probability of observable damage), that the system initially in E_1 will eventually pass to E_a ; also the probability, λ_0 (probability of recovery), that the system will return to E. These probabilities are given by

$$\lambda_a = p_{a-1,a} \cdot \sum_{n=1}^{\infty} p_{1,a-1}^{(n)} = \frac{1}{2^{a-1}} , \tag{2.5}$$

where $p_{1,a-1}^{(0)} = 0$, and

$$\lambda_0 = p_{10} \cdot \sum_{n=0}^{\infty} p_{11}^{(n)} = 1 - \frac{1}{2^{a-1}} , \tag{2.6}$$

where $p_{11}^{(0)} = 1$.

In order for the above results to be useful in experimental radiobiology we will now give several relationships that result from the application of the above model to many organisms. Suppose we plan to check the above model by irradiating N organisms, and wish to obtain an expression for the number of survivors under the assumption that the above model is applicable to each organism. If the organisms do not interact, the probability that there will *eventually* be n_0 survivors out of N organisms will be given by the binomial distribution

$$b(n_0 ; N, \lambda_0) = \binom{N}{n_0} \lambda_0^{n_0} \lambda_a^{N-n_0} . \tag{2.7}$$

If we wish to obtain an expression for the number of survivors after a finite period of time (finite number of steps), we must rewrite (2.7) as

$$b(n_0 ; N, p_{10}^{(n)}) = \binom{N}{n_0} [p_{10}^{(n)}]^{n_0} [p_a^{(n)}]^{N-n_0} ; \tag{2.8}$$

that is, we have replaced λ_0 and λ_a by the n-step transition probabilities $p_{10}^{(n)}$ and $p_{1a}^{(n)}$ respectively. Using well-known relationships we can now find the probability that there will be *at most* n_0 survivors $B(n_0 , N, p_{10}^{(n)})$, in terms of the incomplete beta function

$$B(n_0 ; N, p_{10}^{(n)}) = \sum_{\alpha=0}^{n_0} b(\alpha; N, p_{10}^{(n)}) = 1 - N\binom{N-1}{n_0} \int_0^{p_{10}^{(n)}} \xi^{n_0}(1-\xi)^{N-n_0-1}\, d\xi. \tag{2.9}$$

Equations (2.7), (2.8), and (2.9) are all functions of the number of states a. The number of states has been expressed in terms of the number of nucleotide units in the macromolecule assumed to undergo depolymerisation following a hit, viz., $a = 1$ is tantamount to a macromolecule of 100 nucleotide units, etc. (32).

In an attempt to attach some biological significance to the number of states in the above chain model we have encountered the following problem which may be of interest to statisticians. The problem is concerned with estimating the number of states from experimental observations. Suppose we consider the following experimental situation. Let us irradiate, one at a time, a large number of organisms. Now according to the above model an irradiated organism will either die or recover, hence there are two observable states. Let $t_1^a , t_2^a , \cdots , t_n^a$ be the observed times required for m organisms to die, and let $t_1^0 , t_2^0 , \cdots , t_n^0$ be the observed times required for n organisms to recover. That these states have been reached can be determined by physiological and biochemical tests. Now, given the distribution of the t^a's and the t^0's, together with the transition probabilities between states, what is the maximum likelihood estimate of a? In order to determine an estimate of a it is necessary to make some assumption concerning the time required for each transition between states.

3. *Social behaviour.* In the development of a mathematical biology of social behaviour stochastic methods have played an outstanding role. One of the problems receiving attention is that of dominance relations in social groups. In particular peck-order or "peck-right" phenomena in animal societies has been investigated; peck-order being defined as a binary asymmetric relation between each pair in a finite collection of individuals.

A. Rapoport has developed a theory of peck-order utilising a simple Markov chain model (27). This model is developed as follows: Let S represent the structure of a group of individuals, and denote by p_{ij} the probability of a change from structure S_i to S_j . Obviously p_{ii} represents the probability of preservation of the structure S_i . It is assumed that encounters (or transitions) occur at regular intervals, and this interval is taken as the unit of time. Denote by $S_i(t)$ the probability of occurrence of the structure S_i at time t. Now S_i at time t may have

occurred in either of the two ways given by the relation

$$S_i(t-1) \rightarrow S_i(t) \leftarrow S_j(t-1).$$

The probability of its having come from S_i through no change is $p_{ii}S_i(t-1)$; and the probability of its having arisen from S_j at $t-1$ is $p_{ji}S_j(t-1)$. Therefore the occurrence of S_i at t from any of the possible structures or through no change is

$$S_i(t) = \sum_{k=1}^{n} p_{ki}S_k(t-1), \qquad \text{for all } i. \tag{3.1}$$

If $S(t)$ is the vector $\{S_1(t), S_2(t), \cdots, S_n(t)\}$ equation (3.1) can be written in vector-matrix form

$$S(t) = (p_{ij})S(t-1). \tag{3.2}$$

By iteration we obtain

$$S(t) = (p_{ij})^t S(0). \tag{3.3}$$

Therefore

$$S(\infty) = \lim_{t\to\infty} (p_{ij})^t S(0). \tag{3.4}$$

Rapoport has been able to show that under certain conditions the limit in equation (3.4) is independent of $S(0)$. This means that under those conditions the structure of the society will tend to a form which is independent of the initial structure. This result, which is a familiar one in the theory of Markov chains, tells us that the chain is ergodic.

Recently H. G. Landau (21) has applied the theory of Markov chains to the study of dominance relations and the structure of animal societies when social factors, i.e., those due to the existing or previous social structure, or dominance relations, or to the outcome of previous encounters, are assumed to have some effect.In this study the states E_k are the possible structures of the society, with transition probabilities, p_{ij} , giving the probability of change from E_i to E_j as the result of an encounter. Before going on, we define several terms which will be used later: (1) The *dominance structure* is the matrix $A = (a_{ij})$, with elements $a_{ij} = 1$ if $i > j$ (read i dominates j), $a_{ij} = -1$ if $j > i$, and $a_{ii} = 0$ for $i = 1, 2, \cdots, n$; (2) The *score structure*, V, is the set of n integers $V = (v_1, v_2, \cdots, v_n)$, where v_i means that the i-th member dominates v_i of the others; (3) The *hierarchy* is the structure $V = (n-1, n-2, \cdots, 0)$; (4) The *hierarchy index*, h, is defined as

$$h = \frac{12}{n^3 - n} \sum_{i=1}^{n} \left(v_i - \frac{n-1}{2}\right)^2;$$

hence h lies between 0 and 1, having the value 1 when V is the hierarchy, and 0 when V is the equality, i.e., when $v_1 = v_2 = \cdots = v_n = (n-1)/2$.

Before introducing the effect of social factors, Landau was able to show that under certain conditions the Markov chain whose states are the different structures is ergodic. This is the same result obtained earlier by A. Rapoport. A sufficient condition for ergodicity is that all $\epsilon_{ij}q_{ij}$ must be greater than zero, where ϵ_{ij} is defined as the probability of an encounter between members i and j, and q_{ij} is the probability that $i > j$ after an encounter. This condition implies that in any structure the probability that any dominance relation be either reversed or maintained is not zero, hence $p_{ii} > 0$, and the chain is said to be aperiodic. In addition, since any reversal of dominance relations has non-zero probability, it is possible to go from any structure to any other by a finite number of one-step transitions. This means that the chain is indecomposable. That the chain is ergodic follows by using the well-known ergodic theorem for indecomposable Markov chains with aperiodic states.

The social factors considered and the results obtained were: (1) A uniform bias against reversal of dominance will have no effect on the stationary distribution of the structure of the society; (2) If the probability of dominance is a linear function of the previously established score, there will be a small tendency for the society to move toward the hierarchy. If a member never challenges another whose score exceeds his own by two or more, or if he can never dominate it he should challenge, then the hierarchy is the only stable structure, i.e., it is an absorbing state.

Before closing this section we remark that a generalisation of the above results is possible through the use of models formulated as multiple Markov chains. Here one is able to take into consideration various hereditary effects which may be important in the determination of social structures.

4. *The spread of epidemics and rumours.* Early work in the mathematical theory of epidemics was mainly concerned with the development of deterministic models of the spread of disease through a population. In the deterministic treatment of epidemics the assumption is made that for given numbers of susceptible and infectious individuals and given infection and removal rates, a definite number of new cases would arise in a given period of time. It is known however that many chance factors enter into the determination of the number of infected individuals at any time, thus pointing up the need for stochastic models to supplement or replace deterministic models.

In a recent paper N. T. J. Bailey (3) has considered the use of sto-

chastic models in epidemic theory, and given the stochastic treatment of a simple epidemic. The epidemic process is characterised by the stochastic differential-difference equations

$$\frac{dp_r(t)}{dt} = (r+1)(n-r)p_{r+1}(t) - r(n-r+1)p_r(t),$$

$$(r = 0, \cdots, n-1.) \qquad (4.1)$$

and

$$\frac{dp_n(t)}{dt} = -np_n(t),$$

where $p_r(t)$ is the probability that in a population of n individuals there are r susceptibles still uninfected at time t. The above equation is substantially the same as W. Feller's birth-and-death equation occurring in the theory of population growth.

The concept of a random net can also be applied to the study of epidemics if one assumes the probability of transmission of the disease is the same for each pair of individuals in the population. The weak connectivity of the net now represents the expected number of individuals which will contract the disease eventually, and the strong connectivity represents the probability that the entire population will succumb.

The branching processes discussed in section II, and the random net model can also be used in the study of the spread of rumours. Recently A. Rapoport and L. I. Rebhun (30) have applied the theory of random nets to the theory of rumour spread. Here the weak connectivity of the net appears as the saturation fraction of the number of "knowers" (individuals that have heard the rumour) in a thoroughly mixed population through which the message is diffused, and where each knower tells the message to a finite number of individuals. A method is given for translating the time course equation of rumour spread (where the time is measured in the number of removes from the starters) into an ordinary continuous time equation if the distribution of the telling intervals is known. This study utilised experimental data obtained by S. C. Dodd and his staff (8) at the Washington Public Opinion Laboratory.

We are now considering the use of age-dependent stochastic branching models in the study of epidemics and rumour spread.

5. *Communication nets.* A communication net can be defined as a collection of nodes with channels connecting pairs of nodes in the collection. The use of this definition enables us to consider a communica-

tion net as a random net in which the points and axones have been replaced by nodes and channels.

The structural properties of communication nets have been investigated by using a matrix representation (Ordinary and Boolean matrices have been used) of its structure. The net is represented by a square matrix $A = (a_{ij})$, the elements a_{ij} having the value unity or zero depending on whether or not a channel connects the i-th to the j-th node. The order of A is determined by the number of nodes in the net. Stochastic matrices can also be used to represent net structure; here the matrix elements p_{ij} represent the probability that a channel is between the i-th and j-th nodes, or the probability that the i-th node sends its information to the j-th node.

At the present time we are considering the application of Markov chain techniques to the study of changes in the structure of communication nets. In this approach we must consider a Markov chain whose states are the different possible net structures, hence we have a chain whose states are matrices. A. Shimbel has shown that for a net with n nodes there are less than $N = 2^{n(n-1)}$ possible structures (or states). In addition to these structures we must add another structure whose matrix has elements greater than or equal to unity. This matrix will form an absorbing state since it represents the structure of the net when each node has all of the information originally possessed by the other nodes in the net. The task facing us now is the determination of the transition probabilities p_{ij} . The main difficulty is due to the lack of an adequate measure of matrix structure, and no clue as to what form the transition probabilities might have. One could get some ideas about the latter from experimental studies by utilising the methods of T. W. Anderson, which we referred to in section II.

Recently social psychologists have been using the concept of a communication net in studying problem solving by groups (22). Two cases have been considered: (i) All members of the group can communicate freely with one another, and (ii) the communication patterns are restricted. An experiment designed by psychologists to utilise the results of the Anderson study would be extremely useful in enabling the mathematical biologist to develop a realistic model of changes in communication net structure.

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A METHOD FOR COMPARING FLY-REPELLANT SPRAYS

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Introduction

In a standard technique for comparing two fly-repellant sprays, batches of mice are treated with the two materials under test and are subsequently exposed under anaesthetic to attack by flies of the genus *Stomoxys*. A control untreated batch is similarly exposed for purposes of comparison. If replicate tests are carried out on several occasions, it is found that the attack rates in the control and in the treated mice vary considerably, and the problem arises of finding some stable basis for comparison.

In practice, five mice are used on each occasion for each treatment and for the controls, and each mouse is exposed to attack by 20 flies (this figure is not exact, but the variations in the number of flies are small enough to be neglected). The percentage attack rate is worked out for each mouse, and these rates are then averaged to give three mean attack rates for each occasion of testing. Full biological details of the method will be published elsewhere by Mr. L. C. Stones.

The Mathematical Model

Suppose on one occasion the observed mean attack rates on the controls and in the two treated batches of mice are p_0 , p_1 , and p_2 (expressed as proportions). We may suppose that only a proportion p_0 of the flies were liable to attack in the treated batches, and hence that the proportions actually repelled were $(p_0 - p_1)/p_0$ and $(p_0 - p_2)/p_0$. Thus p_1/p_0 and p_2/p_0 are estimates of the attack rates in the treated mice when the attack rate in the controls is 100%, and it seems reasonable to suppose that these rates are essentially constant and to base our comparisons upon them. If we write p_A , p_B for these "true" attack rates, the observed rates on occasion 1 are estimates of

$$p_{01} \; ; \qquad p_{01} \times p_A \; ; \qquad p_{01} \times p_B$$

and from a set of such observations we wish to estimate p_A and p_B .

It is convenient to transform the observations in order to render the effects additive. This implies taking logarithms. Writing $x = \log p$, the expected values of the observations are given in terms of the parameters by

$$x_{01} \; ; \qquad x_{01} + x_A \; ; \qquad x_{01} + x_B$$

Estimation of the parameters by maximum likelihood assuming binomial distributions leads to rather intractable equations; moreover, the variability between replicate mice on one occasion is considerably greater than that expected in ordinary binomial sampling. However it appears empirically that an observed proportion p is subject to a variance roughly proportional to pq/n, where $q = 1 - p$. It follows that a value x has a variance proportional to q/pn, and on this basis we can estimate the parameters by weighted least squares.

The Method of Estimation

The weights to be used in the calculations should be based on expected rather than observed attack rates, and an iterative procedure will have to be used. Rather than use the observed values to obtain weights for the first cycle of calculations, it is better to start from rough preliminary estimates as otherwise convergence may be rather slow. To obtain these, set out the observed attack rates (as percentages) and their logarithms in a two-way table such as Table 1, which refers

TABLE I. MEAN ATTACK RATES AND THEIR LOGARITHMS AND PRELIMINARY ESTIMATES OF THE CONSTANTS

		O	*A*	*B*	
1	p	60.2	4.0	8.8	
	x	1.780	0.602	0.944	$x_{01} = 1.773$
2	p	55.2	6.8	10.4	
	x	1.742	0.833	1.017	$x_{02} = 1.773$
3	p	41.4	2.8	4.0	
	x	1.617	0.447	0.602	$x_{03} = 1.593$
		1.713	0.627	0.854	
		$x_A = -1.086$		$x_B = -0.859$	

to an actual experiment with three occasions. Working with the logarithms, calculate the means of the columns of this table, and by subtracting the control figure from the other two, obtain estimates of x_A and x_B. The estimates of the control attack rates can be obtained in the same way from the means of the rows, but it is preferable to give relatively higher weights to the control figures since they will usually be more accurately determined. The exact weight chosen is not of great importance; in Table I a weight of 8 has been used which slightly simplifies the arithmetic. We thus take 8 times the control

figure, add the two treatment figures, subtract the estimates of x_A and x_B and divide by 10—for example

$$x_{01} = (8 \times 1.780 + 0.602 + 0.944 + 1.086 + 0.859)/10 = 1.773.$$

From these preliminary estimates we can calculate the weights to be used in the first cycle of least squares adjustment. In Table II

TABLE II. LEAST SQUARES ADJUSTMENT

		O	*A*	*B*	
1	p'	59.3	4.9	8.2	
	w	146	5	9	160
	wx	259.880	3.010	8.496	271.386
2	p'	59.3	4.9	8.2	
	w	146	5	9	160
	wx	254.332	4.165	9.153	267.650
3	p'	39.2	3.2	5.4	
	w	64	3	6	73
	wx	103.488	1.341	3.612	108.441
		356	13	24	393
		617.700	8.516	21.261	647.477

$$x_{01} = -0.03125x_A - 0.05625x_B + 1.69616$$
$$x_{02} = -0.03125x_A - 0.05625x_B + 1.67281$$
$$x_{03} = -0.04110x_A - 0.08219x_B + 1.48549$$

$$12.564x_A - 0.809x_B = -12.782$$
$$-0.809x_A + 22.494x_B = -17.973$$

$\Delta = 281.9601$

$c_{11} = 0.079777 \quad c_{12} = 0.002869 \quad c_{13} = 0.044559$

$x_A = 1.07127 \quad x_B = -0.83753$

$x_{01} = 1.77675 \quad x_{02} = 1.75340 \quad x_{03} = 1.59836$

	d.f.	S.S.	M.S.
Constants	5	1097.8807	
Residual	4	0.4763	0.1191
Total:	9	1098.3570	

we set out the expected attack rates p' calculated from the preliminary estimates, the weights w calculated as $100\ p'/(1 - p')$ and the products

of the weights by the observed x's, together with row and column totals.

The next step is to set up the normal equations. There will be one equation for each constant—here five in all. The coefficient of x_A in the first equation is the total weight of all observations involving x_A; the coefficients of the other x's are the weights of those observations involving these x's as well as x_A , and the right hand side is $\sum wx$ taken over all observations involving x_A . All these quantities are found in the second column of Table II. The other equations are set up in the same way, and the complete set is as follows—

$$\begin{aligned}
13x_A \quad &+ \quad 5x_{01} + \quad 5x_{02} + \quad 3x_{03} = \quad 8.516 \\
24x_B + \quad &9x_{01} + \quad 9x_{02} + \quad 6x_{03} = \quad 21.261 \\
5x_A + \; 9x_B + 160x_{01} \quad &\qquad\qquad\quad = 271.386 \\
5x_A + \; 9x_B \quad &+ 160x_{02} \qquad = 267.650 \\
3x_A + \; 6x_B \quad &\qquad\quad + 73x_{03} = 108.441
\end{aligned}$$

The last three equations give x_{01} , x_{02} and x_{03} in terms of x_A and x_B and by substituting in the first two we get a pair of simultaneous equations to determine x_A and x_B . All the coefficients in the normal equations are to be found in Table II, and with a little practice it becomes unnecessary to write the equations out in full.

If the equations for x_A and x_B are

$$\begin{aligned}
ax_A - bx_B &= h \\
-bx_A + cx_B &= k
\end{aligned}$$

we calculate

$$\begin{aligned}
\Delta &= ac - b^2 \\
c_{11} &= c/\Delta \qquad c_{12} = b/\Delta \qquad c_{22} = a/\Delta \\
x_A &= c_{11}h + c_{12}k \qquad x_B = c_{12}h + c_{22}k
\end{aligned}$$

and x_{01} , x_{02} and x_{03} follow from the last three normal equations. If the values obtained differ markedly from the preliminary values, a second cycle of calculations should be performed, basing the weights on the values of p' calculated from the results of the first cycle. In our example, a second cycle is hardly necessary.

Precision of the Estimates

It may be desired to attach standard errors to x_A and x_B although these will have to be interpreted with caution as the underlying distributions may be rather far from normal. We require for this purpose an analysis of variance. The sum of squares accounted for by the constants is found by multiplying each constant by the right hand

side of the corresponding equation in the last cycle of calculations and summing the products. The total sum of squares is found by multiplying each observed x by the corresponding wx from Table II (or its equivalent in a later cycle if one is carried out) and summing. By subtraction we obtain a residual sum of squares (here with 4 d.f.) and hence the residual mean square, s^2. The variances of the constants are then estimated by

$$V(x_A) = c_{11}s^2 \quad V(x_B) = c_{22}s^2 \quad V(x_A - x_B) = (c_{11} - 2c_{12} + c_{22})s^2$$

This estimate of error is based on very few degrees of freedom, and may be supplemented by an alternative estimate derived from differences within the groups of five mice. Comparison of the two estimates will also provide a test of the goodness of fit of the assumed model. The usual procedure would be to start by transforming the attack rates on individual mice into logarithms, but this is not possible here owing to the occurrence of zero values. Instead we can calculate for each group of five mice an estimated variance of the observed attack rates, $\sum(p - \bar{p})^2$, with 4 d.f. From the values of p' used in the final cycle, we obtain a "theoretical" value $p'(1 - p')/20$. If we sum these quantities over all the groups and take the ratio of the sums, we obtain a "heterogeneity factor" α, such that the variance of an observed attack rate p is given by $\alpha pq/n$. To render this comparable with the estimate obtained from the analysis of variance we must multiply by $(\log_{10}e)^2$ to allow for the fact that common logarithms have been used. The "within groups" estimate of error variance is thus 0.1887 α, and this can be used in place of the "between groups" estimate unless the latter is substantially the greater of the two. In our example the sum of the empirical within-group variances was 833.0 while the sum of the theoretical variances is 530.0, so that $\alpha = 1.57$ and $s^2 = 0.296$. Using this estimate of variance we can summarise the results in the form

$$x_A = -1.071 \pm .154$$

$$x_B = -0.838 \pm .115$$

$$x_A - x_B = -0.234 \pm .187$$

In general, the control groups will receive a higher weight than the treated groups since they give a higher attack rate. It would thus be preferable to allocate more mice to each treated group than to the controls. This is easily allowed for in evaluating the weights.

I am grateful to Mr. L. C. Stones of the Cooper Technical Bureau, Berkhamsted, for proposing this problem and for allowing me to use the data from his experiments.

AN EXAMPLE OF THE USE OF FRACTIONAL REPLICATION[1]

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Introduction

The principles of fractional replication have been laid down by Finney (1,2) and Kempthorne (3,4). These principles are of considerable utility in exploratory research work, when the research worker has to consider a number of factors in combination, but is clearly unable to test the full factorial set.

The purpose of this paper is to illustrate the use of these principles in what may be regarded as a complex situation. The problem to which this application has been made was the determination of various aspects of the dehydration of sweet corn.

The Problem

The production of dehydrated sweet corn ordinarily includes a consideration of:

(1) Factors of growth
(2) Varietal characteristics
(3) Stage of harvesting
(4) Method of preparation of raw products
(5) Method of Blanching
(6) Method of Dehydration
(7) Conditions of storage
(8) Evaluation of the final product

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These factors may be conveniently divided into three groups dealing with growth, manufacture and acceptability of the product.

Since there are only about eight golden varieties of sweet corn which are normally grown in mid-western states, the experimental procedure was arranged to include all eight varieties. Because a comparison of varieties for dehydration could only be made taking account of the remaining important variables in the dehydration procedure, this work was set up to include consideration of varieties, field replications, blanching, dehydration and storage.

Obviously this list does not include all of the variables which might be considered to have an effect on the final product, but it was thought that these variables represent the smallest number of the most important variables which should be considered in one experiment.

Sweet corn is ordinarily harvested at a moisture content of 65 to 75 percent for any processing operation and this includes the operation of dehydration. It has been found that sweet corn is most acceptable when harvested in this range of maturity. Following harvesting and preparation, the corn kernels must be blanched to inactivate the enzymes contained in the kernel which, should they remain active, would cause a loss in quality generally, and specifically the development of off-flavors in the corn. The operation of blanching includes the application of a temperature level for a given length of time sufficient to inactivate the enzymes which cause loss of quality. In the dehydration procedure, drying is carried out by one of several means in a current of relatively dry air until the corn has reached a moisture content, usually around five percent, which has been found desirable for maintaining the quality of the product during subsequent storage. Following dehydration, the product may be packaged in a variety of ways, again in an attempt to maintain quality. In addition to packaging, the temperature of storage and the duration of storage both exert effects upon the ultimate quality of the product. In the light of these observations, it appears to be necessary to make any comparison of the quality of sweet corn obtained from one of another variety in terms of the processing operations, their intensity and extent.

Since adequate objective tests are not available as criteria of quality of dehydrated corn, among other food products, it was necessary to use a taste panel to evaluate the final product. These evaluations were made in terms of appearance, odor, flavor, texture and color by three experienced judges.

The particular aspect of the whole process which prompted the present investigation was the need for information to indicate the suitability of the varieties considered for dehydration. As has been pointed

out, no comparisons of these varieties could be made without consideration of the manner of preparation, and an apparently simple question has led us into the necessity of considering a rather complex one, analogous to Fisher's discussion (5) of the desirability of factorial experimentation.

From the point of view of experimental design the problem was resolved into finding a method for the inclusion of all the factors mentioned in a design. It seems impractical to examine all these factors exhaustively in a single experiment, and an exploratory experiment was therefore considered which would indicate the degree of importance of the various factors.

The Structure of the Experiment

Given the above factors which are to be incorporated in the experiment, it is necessary to decide the ways in which these factors are to be allowed to vary, the number of levels of each and so on. The decisions on these points are of course partially based on subject matter knowledge, but must take into account the difficulties which may arise in the statistical design. For example, the authors know of no way of performing a $5 \times 3 \times 2$ design under reasonable assumptions except in complete replicates.

The 2^n factorial system is particularly suitable for fractional replication. In fact fractional replication is not useful with mixed factorial systems and the 3^n system leads rapidly to an unduly large number of treatment combinations, since a 1/3 replicate is advisable only with 5 or more factors. In view of these facts and the general exploratory nature of the study, it was therefore decided to work within the 2^n system. The 2^n system has a further advantage that it is possible to include factors at 4 or 8 levels (in fact any power of 2).

As mentioned earlier, eight varieties of golden sweet corn were included. Four dates of harvesting based on the moisture content of the maturing kernels were used. In the blanching operation two combinations of time and temperature were considered while in the dehydration operation, temperature was controlled at two levels, the duration being what was necessary to reduce the moisture to a chosen level. This procedure was used in an attempt to discover something about the effect of these temperature combinations on quality of the final product. The temperatures of storage and lengths of storage were chosen to cover a reasonably wide range.

Each of the factors with a number of levels equal to a power of 2 may be represented by a suitable number of pseudofactors each with 2 levels. The resulting factors may therefore be represented as follows:

varieties:	a, b, c
dates:	d, e
field replicates:	r, s
time-temperature of blanching:	f
temperature of dehydration:	g
temperature of storage:	h
length of storage:	j

and formally we have a 2^{11} experiment, that is, one with 11 factors each at 2 levels. The particular way in which the levels of the pseudofactors correspond to the levels of the factors is unimportant except for purposes of identification.

The next step in the construction of a design is the choosing of a suitable fractional replicate and this is done by choosing an appropriate identity relationship. With 11 factors, some of which are pseudofactorially related as in the above, it will be found that a 1/4 replicate of the full 2048 treatment combinations can be chosen in such a way that no interactions of two factors (*not* pseudofactors) are mutually confounded.

The choice of identity relationship depends on the experimental limitations. With the particular resources available, 4 plots for each variety were possible, and as far as varieties are concerned the plan necessarily consisted of 4 randomized blocks of 8 plots. A completely randomized design as regards varieties could have been used, but was expected to be less efficient. These plots were to be divided into 4 split-plots for the dates, and then each split-plot divided into 4 parts or split-split plots for the other treatments. With a fully replicated experiment each split-plot would have been divided into 16 parts for the other treatments, but this would have led to the impossibly large total number of treatment combinations. What was used amounts then to a combination of split-split-plot confounding and fractional replication. A design of essentially different structure would have been impossible.

The identity relationship actually used is the following:

$$I = ADRFG = BESHJ = ABDERSFGHJ.$$

This identity relationship specifies the treatment combinations to eb tested in the following way: let $x_1, x_2, \cdots, x_{11}$ correspond to the factors or pseudofactors a to j, in the order listed above, and let x_1 equal 0 if factor a is at the lower level and x_1 equal 1 if factor a is at the upper level and likewise for $x_2, x_3, \cdots, x_{11}$. Then the treatment combinations tested are those for which both

$$x_1 + x_4 + x_6 + x_8 + x_9 \text{ is even}$$

and $$x_2 + x_5 + x_7 + x_{10} + x_{11} \text{ is even}$$

The consequences of using only the selected subset of the treatment combinations may be written down easily from the identity relationship. Thus multiplying the whole relationship by A and imposing the rule of replacing A^2 by unity, we get

$$A = DRFG = ABESHJ = BDERSFGHJ.$$

This says that with the selected subset of treatment combinations the main effect of factor a is completely confounded with the four factor interaction $DRFG$, the five factor interaction $ABESHJ$ and the nine factor interaction $BDERSFGHJ$.

Similarly we have

$$AD = RFG = ABDESHJ = BERSFGHJ.$$

It may be verified that no two factor interactions are mutually confounded; where for example AD, $ABCDE$ are both in fact two-factor interactions, each being a single degree of freedom contrast in the total of 21 degrees of freedom for varieties by dates.

The above may appear somewhat obscure to some readers, and it may help them to discuss briefly a fractional design which we would not have used. Consider the identity relationship

$$I = ABDEF = CDEGH = ABCFGH.$$

The consequences of this relationship may be traced out as before. Thus for example

$$A = BDEF = ACDEGH = BCFGH$$

and so on, so that main effects are confounded with four and five factor interactions. However, we also find

$$ABCF = CDEA = ABDEFGH = GH$$

so that $ABCF$, which is in fact a one degree of freedom contrast from the interaction of varieties and time-temperature of blanching, is completely confounded with the two-factor interaction GH.

The enumeration of the 512 treatment combinations which are to be tested with the chosen identity relationship is most easily made with I.B.M. equipment. First it is necessary to construct the 512 pseudo-factorial combinations. In the above instance we may write out the 16 combinations on a, d, r, f and g which are even with respect to

ADRFG, that is, the 16 combinations on *a*, *d*, *r*, *f* and *g* which have zero, 2 or 4 letters in common with *ADRFG*, and the 16 combinations on *b*, *e*, *s*, *h*, *j* which are even with respect to *BESHJ*. From these we generate all the possible 256 combinations of the two sets of 16, and then we take these 256 combinations both with and without *c*. All these operations are done with I.B.M. equipment, presence being indicated by a "1" in the appropriate column and absence by a "0". Having obtained the 512 pseudofactorial combinations we may then recode to the original factors by sorting in master cards and gang punching.

The randomization procedure was the standard one for split-split plot designs.

Analysis of the Experiment

As we have indicated in the introduction, the characteristics of interest were subjective observations on the samples of corn, obtained from the cans after the designated length and temperature of storage. These observations were made by three workers in Food Technology at Iowa State College. Information would be of the most value, of course, when obtained from a sample of some specified population of tasters but in the absence of such a defined population, it appeared best to use available staff with previous experience in the area of investigation. Inferences which are drawn from the experimental data must therefore be restricted to the judges actually used and analyses were based consequently on the total responses for the three judges.

The structure of the analysis of variance is determined by two considerations:

(1) the assumptions made in using the identity relationship (namely that interactions of three or more factors were negligible) and
(2) the restrictions used in the randomization.

The analysis of variance as in all cases of split-split-plot designs, consists of three portions:

(1) the analysis of whole plot totals
(2) analyses within whole plots between split-plot totals and
(3) analyses within split-plots between split-split plots.

This analysis of variance is based on the assumption that all interactions involving three or more factors are zero. The only part of this partition which merits comment is the inclusion of *FG* and *HJ* in the split-plot portion and not in the split-split-plot portion. This comes about because from the identity relationship we obtain

TABLE 1. PARTITION IN ANALYSIS OF VARIANCE OF EXPERIMENT

	df
Replicates (R, S)	3
Varieties (A, B, C)	7
Whole-plot error	21
Dates (D, E)	3
Dates × varieties	21
FG	1
HJ	1
Split-plot error	70
F, G, H, J	4
FH, FJ, GH, GJ	4
Varieties × F, × G, × H, × J	28
Dates × F, × G, × H, × J	12
Split-split-plot error	336
Total	511

$$FG = ADR = BESFGHJ = ABDERSHJ$$

so that FG is completely confounded with ADR which is a component of the split-plot error, in fact, being a component of varieties × dates × replicates. The same reasoning holds for HJ.

Results of the Experiment

It is not our purpose to review here at all extensively the result of the experiment. We shall instead present the analysis of variance for one of the characteristics of interest and a few summary tables with estimated standard errors. Our sole purpose is to show that the pattern of experimental observations was effective for the purposes of a preliminary overall investigation.

As mentioned previously the experimental units were measured subjectively, and a scale of 0 to 9 was used. The analysis of variance given in Table 2 for the appearance of the resulting corn is typical.

From this analysis of variance, we see that the experiment demonstrated effects due to varieties, dates and their interactions and to the storage treatments, but that the blanching and dehydration treatments were almost ineffective in changing the characteristic. There is a little evidence for an interaction of the blanching and dehydration treatments.

As an example of effects of treatments, the following was found for varieties and dates with regard to appearance:

TABLE 2. ANALYSIS OF VARIANCE FOR APPEARANCE

Source of variation	Degrees of freedom	Sum of Squares	Mean Squares
Rep	3	38.71	
Var	7	1,190.19	170.03**
Error *A*	21	635.23	30.25
Total	31	1,864.13	
Date	3	9,046.14	3,015.38**
Date × var.	21	1,502.80	71.56**
FG	1	116.28	116.28**
HJ	1	578.00	578.00**
Error *B*	70	2,787.96	39.83
Total	127	15,895.31	
F	1	36.12	36.12
G	1	12.50	12.50
H	1	5,189.26	5,189.26**
J	1	2,601.01	2,601.01**
FH	1	.94	.94
FJ	1	29.07	29.07
GH	1	23.63	23.63
GJ	1	1.76	1.76
Var × *F*	7	51.32	7.33
Var × *G*	7	146.63	20.95
Var × *H*	7	418.62	59.80*
Var × *J*	7	722.62	103.23**
Date × *F*	3	61.59	20.53
Date × *G*	3	39.02	13.01
Date × *H*	3	467.82	155.94**
Date × *J*	3	141.98	47.33
Error *C*	336	7,303.68	21.74
Total	511	33,142.88	

A discussion of the utility of the experimental results is given by Tischer et al (6). It is sufficient to note that the experiment served its purpose in picking out clearly factors which are important, and in establishing the existence of some interactions.

TABLE 3. VARIETY AND DATE EFFECTS AND INTERACTIONS

Variety	Date				Mean
	1	2	3	4	
1	2.56	3.59	4.45	5.00	3.90
2	3.19	4.23	4.53	5.10	4.26
3	3.51	5.04	5.17	4.92	4.66
4	3.09	4.34	4.68	5.28	4.35
5	2.78	3.92	5.08	5.01	4.20
6	3.24	3.89	4.97	5.17	4.32
7	3.63	3.50	4.05	3.95	3.78
8	3.08	4.06	4.83	5.07	4.26
Mean	3.14	4.07	4.72	4.94	4.22

Standard errors: of variety means: 0.12
of date means: 0.09
of entries in table for interaction: 0.26

Average Effects of Blanching, Dehydration and Storage

	Blanching temperature	Dehydration temperature		Storage		Mean
				3 months	6 months	
Low	4.26	4.24	70°F	5.30	4.19	4.75
High	4.17	4.19	100°F	3.88	3.49	3.69
				4.59	3.84	

The standard error of means for blanching temperature, dehydration temperature, storage time and storage temperature is 0.05.

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A STATISTICAL DESIGN FOR THE EFFICIENT REMOVAL OF TRENDS OCCURRING IN A COMPARATIVE EXPERIMENT WITH AN APPLICATION IN BIOLOGICAL ASSAY

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SUMMARY

The method of construction of a certain class of designs for use with quantitative factors by means of which a trend occurring during a comparative experiment may be eliminated without loss of efficiency is indicated. The design and analysis is illustrated with an example from biological assay.

1. INTRODUCTION

In recent work (Box and Wilson, 1951; Box, 1952) it has been emphasised that, when dealing with quantitative factors, (i. e. factors like temperature, time, dose, which can be varied on a continuous scale) it is frequently advantageous to employ statistical designs which depart from the "factorial" principle. It has long been appreciated that it is the *orthogonal* property of designs which produces efficiency. The factorial method, in which are tested all (or a selected fraction) of possible combinations of a few levels of each of the factors, is often a convenient way of obtaining orthogonality but it is, of course, by no means the only way, nor is it, in all circumstances, the 'best' way. Designs which may be used to determine the effects of a number of factors but which are not necessarily factorial designs or parts of factorial designs may conveniently be called *multi-factor* designs.

When we are dealing with quantitative factors if we do not limit ourselves to designs involving a few equally spaced levels of the factors, a great gain in flexibility results and a widening of the possibilities for developing designs for specialised needs. Furthermore, there is no

very great increase in labour in the analysis of the experiment, and arrangements involving fewer observations often result. For example, in the first of the papers referred to above "composite" multi-factor designs of second order, that is to say designs which allowed the determination of all the first and second order effects (linear effects, linear × linear interaction and quadratic effects) were developed which were at least as efficient as the orthodox three-level factorials but required many fewer experiments. In the second paper first order multi-factor designs of maximum efficiency for determination of the linear effects of any number of factors were described. These latter designs allowed simultaneous elimination of trends in the observations (e.g. time trends) without loss of efficiency, whilst a procedure called "angular randomisation" rendered subsequent statistical tests exact whatever the distribution of the observations and whether the assumed mathematical model was exactly realised or not.

Work on a related problem has been published recently by Cox (1951) whose designs retain the factorial principle, but in certain circumstances still allow the efficient elimination of time trends. The purpose of the present paper is to develop a further design of the multi-factor type which makes possible the efficient comparison of two response curves, when a time trend in the results is simultaneously occurring and to show its application in a problem of biological assay.

2. EXAMPLE

A design of this type was used in the biological assay of *d*-tubocurarine chloride. It was required to compare the estimated positions, slopes and curvatures of the dosage-response curves for a test material and a standard material whilst simultaneously eliminating, without loss of efficiency, any time trend which occurred in the course of the assay.

2.1 *Description of the Assay*

For this assay the phrenic nerve of a rat is dissected out and connected to a pointer which records on a smoked drum. This nerve which is immersed in Ringer Solution is given an electric stimulus every 12 seconds which produces a contraction. To obtain an accurate measurement the apparatus is allowed to operate under the same conditions for a period, the average recorded contraction being the value used. When a suitable dose of *d*-tubocurarine is added, a reduction of the contraction occurs. This reduction measured as a percentage is called the *percent inhibition* and is the response used to assay the drug. The apparatus is thoroughly washed out and a second dose is tested. In

this way series of graded doses are given for the test and standard materials and the corresponding responses are measured. From these observations dosage response lines may be constructed from which the relative potency of the test material in terms of the standard may be calculated.

2.2 *Occurrence of Time Trend*

Unfortunately, as the test proceeds it is observed that the response (i.e. the % inhibition) becomes steadily larger due to tiring of the phrenic nerve, incomplete washing, or some other external cause not fully understood. Partial elimination of this trend could be attained, of course, by the use of a randomised block or an incomplete block design, the blocks being successive periods of time. Unless very small blocks could be employed, however, the error variance would be inflated by trends within the blocks, and an alternative method seems desirable.

2.3 *Principle of the Present Design*

In the procedure we adopt, the block size is reduced to two, that is, the experiments are performed in pairs. In the first pair a dose D_1^* of the test preparation is assayed against the same dose D_1 of the standard preparation. In the second pair of experiments the test preparation is assayed against the standard preparation at a dose D_2 and so on until k pairs of doses have been given. The order in which the preparations are tested *within* each pair is random.** We assume that the time trend which occurs during the experiment can be represented by a polynomial of degree p fitted to the block means. The problem is to choose the dose levels so that this trend can be eliminated without any loss of efficiency in the estimation of the effects. This is accomplished by so arranging the doses that the effects to be estimated are orthogonal to the estimated coefficients of the fitted polynomials. If desired, the trend fitted to the pair-means may then be used to eliminate trend effects within the pairs which might otherwise inflate the error.

For the sake of clarity we shall show first how such the design may be used in practice, leaving the method of derivation to §4.

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*In practice of course the test preparation is suitably diluted so that equal quantities of test and standard preparations give approximately equal responses. It would, therefore, be more exact to say that the doses in each pair were proportional rather than equal.

**Here we have assumed that the order is completely random. An alternative procedure which may be adopted when k is even and which has certain advantages is to randomise subject to the condition that in $(\frac{1}{2})k$ of the pairs the test preparation is assayed first and in the remainder the standard preparation is assayed first.

2.4 *Levels of Dose Used*

In the design used for this particular assay eight pairs of doses were used as shown in Table 1. It was expected from previous experience that the response/log dose relationship would be linear for both test and standard, that the lines would be parallel, and the increase in response with time would be roughly linear and could be adequately represented by, at most, a quadratic polynomial. Provision was made in the design used to check these postulates, and less restrictive assumptions than those implied above were actually adopted. In fact effects one degree higher than those expected were allowed for, and it was arranged that:

i) Only four distinct dose levels were employed.
ii) Quadratic response/log dose lines could be fitted if necessary.
iii) A cubic polynomial could be fitted to the time trend.
iv) Differences between test and standard preparations in the dose and time trend curves could be detected. (i.e. interaction effects between samples and the dose and time effects could be estimated).
v) All the effects listed above were orthogonal one with another.

It is shown in §4 that, assuming the doses to be given at equally spaced intervals of time, one suitable arrangement is obtained if the log dose level are arranged so that the deviations from the mean log dose levels are proportional to the numbers, 0.55500, −1.50206, 1.17615, −0.22909, −0.22909, 1.17615, −1.50206, 0.55500, the doses being administered in the order indicated. This means that the actual log levels should be $X_1 = m + 0.55500\ \sigma$, $X_2 = m - 1.50206\ \sigma$, $\cdots$, $X_8 = m + 0.55500\ \sigma$ where m and σ are arbitrary constants so chosen that the design covers the required region of dose levels. It will be noted that, as mentioned above, only four distinct dose levels are used.

Although, of course, the dose of drug cannot be administered in practice to the five-decimal accuracy given above, the error in administering the doses should be no more than that involved in attempting to administer the integer levels usually required by the orthodox type of design and we shall retain the full five-decimal accuracy in our calculations to avoid introducing computational inaccuracies.

In the particular assay described the dosage region of interest lay between 200γ and 270γ. The corresponding region of log dosage is from 2.301 to 2.437. It is found by trial that by taking $m = 2.370$ and $\sigma = 0.050$ a suitable coverage is obtained and we have for the four log dose levels: 2.398, 2.295, 2.429, 2.359 corresponding to actual dose

levels of 250γ, 197γ, 268γ and 228γ to the accuracy attainable. These doses of test and standard were, therefore, administered at equally spaced time intervals in the order shown in Table 1, the order within pairs of test and standard preparations at each dose level being decided by the toss of a coin.

TABLE 1. EXPERIMENTAL PLAN SHOWING ORDER IN TIME OF DOSE LEVELS AND RESPONSES OBSERVED.

Experiment No.	Dose	Test t Standard s	Response Observed (% inhibition)
1	250	s	50.7
2		t	56.4
3	197	s	30.9
4		t	37.5
5	268	t	63.1
6		s	59.9
7	228	t	52.1
8		s	48.7
9	228	s	47.6
10		t	54.3
11	268	t	67.8
12		s	64.3
13	197	t	41.4
14		s	37.2
15	250	t	63.3
16		s	57.6

3. ANALYSIS OF THE RESULTS

The constants required in the calculation are set out in Table 2 whilst the analysis of variance for the data is shown in Table 3. The total crude sum of squares based on sixteen degrees of freedom is entered in the first row of Table 3 and this is then split into two parts each based on eight degrees of freedom and accounting for the variation 'between pairs' and the variation 'within pairs' respectively. The former is the crude sum of squares calculated from the pair-sums, denoted by y_s, shown in column vi of Table 2, and the latter the crude sum of squares

calculated from the pair-differences (test minus standard) denoted by y_d , shown in column vii of Table 2. The correction for the mean is next calculated in the usual way for both sums and differences. In the case of the differences the correction for the mean is in fact the sum of squares due to the difference in average response between the test samples and the standard samples and is called "samples" in Table 3. The corresponding entry in the effect column is the difference in mean response for test and standard samples. Sums of squares calculated direct from the sums or differences must, of course, be divided by two before entering in Table 3.

The two sets of seven degrees of freedom remaining after elimination of the means are now further analysed using the sets of constants given in Table 2.

In columns i), ii), and iii) of Table 2 are shown the values (as they are given by Fisher and Yates, 1942) of the orthogonal polynomials for calculating the linear, quadratic, and cubic time effects. In our notation these are denoted by t_1 , t_2 , and t_3 . Columns iv) and v) show the orthogonal polynomials d_1 and d_2 for calculating the linear and quadratic dose effects. The method of arriving at the levels d_1 and d_2 is explained in §4. They are such that besides having zero sum of products with each other they also have zero sum of products with t_1 , t_2 , t_3 , thus all the effects to be calculated are orthogonal and may be computed independently.

For example, by taking $\sum d_1 y_s$, the sum of products between d_1 and y_s and dividing by $\sum d_1^2 = 8$, the sum of squares for d_1 , we have an estimate of the *sum* (test + standard) of the slopes of the response/log dose curves. By taking the sum of products $\sum d_1 y_d$ of d_1 with the differences y_d and dividing by $\sum d_1^2 = 8$, we have an estimate of the *differences* (test − standard) of these slopes. If, as is expected, the slopes for the test and the standard preparation are the same, then half the former quantity will provide the estimate for the common slope. On the other hand, if a discrepancy in slopes is found then the individual slopes for test and standard may be found from the estimates of the sum and differences. The corresponding sums of squares for the analysis of variance are given by half the square of the sum of products of d_1 and y_s (or y_d) divided by the sum of squares of d_1 . The factor of a half appears because y_s and y_d are each calculated from two observations. In general, we may calculate all the effects and the appropriate sums of squares in this way from the two simple formulae

i) Sum (or difference) of effects $= \sum xy / \sum x^2$

ii) Sum of squares $= (\sum xy)^2 / 2 \sum x^2$

where x is t_1 , t_2 , t_3 , d_1 or d_2 and y is y_s or y_d .

TABLE 2. ORTHOGONAL POLYNOMIALS FOR TIME TREND AND DOSE, WITH SUMS, DIFFERENCES AND CORRECTED DIFFERENCES OF PAIRS OF OBSERVATIONS.

(i)	(ii)	(iii)	(iv)	(v)	(vi)	(vii)	(viii)
Time Trend			Dose		Response		
Linear t_1	Quadratic t_2	Cubic t_3	Linear d_1	Quadratic d_2	y_s Sums (Test + standard)	y_d Uncorrected Differences (Test − standard)	y_c Corrected Differences (Test − standard)
−7	7	−7	0.55500	−0.46956	107.1	5.7	4.65
−5	1	5	−1.50206	0.65424	68.4	6.6	5.55
−3	−3	7	1.17615	0.85467	123.0	3.2	4.25
−1	−5	3	−0.22909	−1.03933	100.8	3.4	4.45
1	−5	−3	−0.22909	−1.03933	101.9	6.7	5.65
3	−3	−7	1.17615	0.85467	132.1	3.5	4.55
5	1	−5	−1.50206	0.65424	78.6	4.2	5.25
7	7	7	0.55500	−0.46956	120.9	5.7	6.75
Divisor 168	168	264	8	4.91837			

TABLE 3. ANALYSIS OF VARIANCE (SUMS)

Source of Variation		Effect	Sum of Squares	D/F	Mean Squares
Grand Total			45,136.26	16	
BETWEEN PAIRS		(Effect Sums) $t + s$			
Total			45,033.70	8	
Correction for mean			43,347.24	1	
Time	Linear	1.048	92.19	1	92.19***
	Quadratic	−0.213	3.81	1	3.81
	Cubic	−0.081	0.87	1	0.87
Dose	Linear	19.917	1586.75	1	1586.75***
	Quadratic	−0.718	1.27	1	1.27
Residual			1.57	2	0.79
Error (1)			3.71	4	0.93

TABLE 3. ANALYSIS OF VARIANCE (DIFFERENCES)

WITHIN PAIRS (uncorrected)	(Effect Differences) $t - s$			
Total		102.56	8	
Samples	4.875	95.06	1	95.06***
Samples × Time Linear	−0.046	0.18	1	0.18
Samples × Time Quadratic	0.119	1.19	1	1.19
Samples × Time Cubic	0.000	0.00	1	0.00
Samples × Dose Linear	−0.541	1.17	1	1.17
Samples × Dose Quadratic	−0.622	0.95	1	0.95
Residual		4.01	2	2.01
Error (2)		4.96	4	1.24
WITHIN PAIRS (Corrected for trend)	**(Effect Differences) $t - s$**			
Total		104.16	8	
Samples	5.005	100.20	1	100.20***
Samples × Time Linear	0.022	0.04	1	0.04
Samples × Time Quadratic	0.100	0.85	1	0.85
Samples × Time Cubic	0.046	0.28	1	0.28
Samples × Dose Linear	−0.388	0.61	1	0.61
Samples × Dose Quadratic	−0.441	0.48	1	0.48
Residual		1.70	2	0.85
Error (3)		2.46	4	0.62

Proceeding in this way the sums and differences of effects due to linear, quadratic and cubic time trends, and linear and quadratic dose effects and their accompanying sums of squares are calculated and are entered in Table 3. Two residual degrees of freedom for the between pairs comparison and two residual degrees of freedom for the within pairs comparisons remain. On comparing the effect mean squares in the table with the appropriate residual mean square it is at once apparent that there is no reason to doubt the postulates, (in mind when the ex-

periment was designed) that a quadratic time trend and linear response/log dose curves would prove adequate to represent the time and dose effects. The sum of squares due to cubic time effects and quadratic dose effects were, therefore, combined with the residuals to give error (1) and error (2) each an appropriate estimate of error for the effects in the corresponding part of the table and each having four degrees of freedom. Significance of the effects when compared with the appropriate error mean square is denoted by asterisks. (Three asterisks denote significance at the 0.1% level).

From the between pairs analysis it is seen that there is a large time trend effect almost completely accounted for by the linear component. There is also a slight suggestion of a quadratic effect but this is not sufficiently large to be definitely established.

From the within pairs analysis we see that there is a real difference in potency between the test and standard preparation, but there is no evidence of difference in slope or curvature for the time trend lines or response curves.

3.1 *Elimination of Trend Effect Within Pairs*

We have seen that a large linear time tread is occurring and that consequently some of the error within groups is due to the change in the level of response occurring in the interval between successive tests within pairs. The doses are spaced (see column (i) of Table 2) so that one unit of time elapses between the first test and the second, and the difference (second test minus first test) is therefore overestimated by an amount β, where β denotes the regression coefficient of the common linear time trend for test and standard preparations. It follows that the variance of the difference (test minus standard) is $2\sigma^2 + \beta^2$ and therefore that error (2) estimates $\sigma^2 + \frac{1}{2}\beta^2$. We may correct for the trend within-pairs therefore by adding or subtracting an amount b from the eight differences, where $b = 0.524$ is an estimate of β and is given by $\frac{1}{2} \times (1.048)$ taken from Table 3. It is subtracted from those pairs where the test preparation was assayed after the standard preparation and added in the contrary case. The "corrected" difference computed in this way to two-decimal accuracy and shown in column (viii) of Table 2 is denoted by y_c. After correction the difference (second test minus first test) is overestimated by an amount $\beta - b$. It follows that the variance of the corrected difference (test minus standard) is $2\sigma^2 + \sigma^2(b)$, where $\sigma^2(b)$ is the variance of b. Consequently, error (3) estimates $\sigma^2 + \frac{1}{2}\sigma^2(b)$. Comparing this with the previous expression we see that it will probably be advantageous to correct for the discrepancy due to trend within pairs if $b > \sigma(b)$. The analysis of the corrected differences given in Table 2,

is carried in a manner exactly similar to that already described for the uncorrected differences.

Now $\sigma^2(b) = \sigma^2/(2 \sum l_1^2) = \sigma^2/336$. Hence the mean square for error (3) calculated from corrected differences estimates $(1 + 1/672)\sigma^2$. The mean square for error (3) multiplied by 672/673 is thus an unbiased estimate of σ^2. It will be seen (as would be expected if the mathematical model assumed were adequate) that estimates of σ^2 from error (1) and from error (3) are compatible and a final combined estimate s^2 based on eight degrees of freedom may be calculated from the combined sums of squares.

{sum of squares for error (1)}

$+$ {672/673 $\times$ sum of squares for error (3)}

Dividing by eight we have the estimate of the combined error variance $s^2 = 0.70$.

3.2 *Calculation of Relative Potency*

If l is the estimated difference (in units of the design) between dose levels of test and standard preparations giving the same response, e is the estimated common slope of the log dose/response curves and a is the average corrected difference in response then

$$-l = \frac{a}{e} = \frac{5.085}{9.959} = 0.503$$

In log dose units, that is $0.503 \times 0.050 = 0.025$. Now antilog $0.025 = 1.059$ whence the estimated relative potency of test to standard preparation is 105.9%.

3.3 *Fiducial Limits for the Relative Potency*

Fiducial limits for the relative potency may now be found following Feiller (1940). We have $a = \bar{y}_c = \bar{y}_d + 2b/8$ (since the correction is subtracted in three cases and added in the remaining five) and $\bar{y}_d$ and b are distributed independently, whence

$$V(a) = V(\bar{y}_d) + \frac{1}{16} V(b) = \left(\frac{1}{4} + \frac{1}{16} \cdot \frac{1}{336}\right)\sigma^2 = \frac{1345}{5376}\sigma^2,$$

$$\text{also } V(e) = \frac{1}{16}\sigma^2.$$

Since a and e are distributed independently and (we shall assume) normally with variances $V(a)$ and $V(e)$ it follows that in repeated sampling

$$z = a + \lambda e$$

is distributed normally about zero with variance

$$V(a) + \lambda^2 V(e) = \left\{\frac{1345}{5376} + \lambda^2 \frac{1}{16}\right\}\sigma^2 = \left\{\frac{1345 + 336\lambda^2}{5376}\right\}\sigma^2$$

and that

$$t = \frac{a + \lambda e}{\left\{\frac{1345 + 336\lambda^2}{5376}\right\}^{1/2} \times s}$$

is distributed as Student's t with the same number of degrees of freedom as s (eight in this example).

Any suggested hypothetical value for λ would, therefore, be rejected at the 100 α% level of significance unless

$$(a + \lambda e)^2 < \left\{\frac{1345 + 336\lambda^2}{5376}\right\} s^2 t_\alpha^2$$

where t_α is the 100 α% level of significance of the t distribution. The 95% fiducial limits are provided, therefore, by those values of λ which just make the above inequality untrue i.e. which make it an equality.

Substituting the numerical values for a, e, s, and t_α and multiplying both sides of the equality by 5376 we have the quadratic equation in λ

$$531{,}895\lambda^2 - 535{,}904\lambda + 129{,}660 = 0$$

which has the solutions

$$\lambda = 0.6038 \qquad \text{and} \qquad \lambda = 0.4037$$

Multiplying these solutions by 0.050 to reduce them to log dose units and taking antilogs we have the 95% fiducial units for potency of 107.2% and 104.8%.

4. CONSTRUCTION OF DESIGNS

Suppose that we are to give k pairs of doses and that a polynomial in time (t) of p-th degree will adequately represent the time-trend and a polynomial in dose level (d) of q-th degree will adequately represent the dosage-response curve in the absence of the time-trend; then provided $p + q$ is less than k we may take as the model.

$$\eta = \beta_0' + \beta_1' t + \cdots + \beta_i' t^i + \cdots + \beta_p' t^p + \alpha_1 d + \cdots + \alpha_i d^i + \cdots + \alpha_q d^q \qquad (1)$$

where η is the true response at dose d and time t.

Now instead of considering powers of t consider the orthogonal polynomials (see for example Fisher and Yates, 1942) which will be denoted by $t_1, \ldots, t_i, \ldots, t_p$ corresponding to $\xi_1, \ldots, \xi_i, \ldots, \xi_p$ in the Fisher and Yates notation. Equation (1) can then be written in the form

$$= \beta_0 + \beta_1 t_1 + \cdots + \beta_i t_i + \cdots + \beta_p t_p + \alpha_1 d + \cdots + \alpha_i d^i + \cdots + \alpha_q d^q \qquad (2)$$

The orthogonal polynomials for equally spaced intervals* and $k = 8$ are given in Table 4.

TABLE 4. ORTHOGONAL POLYNOMIALS FOR $k = 8$.

t_1	t_2	t_3	t_4	t_5	t_6	t_7
−7	7	−7	7	−7	1	−1
−5	1	5	−13	23	−5	7
−3	−3	7	−3	−17	9	−21
−1	−5	3	9	−15	−5	35
1	−5	−3	9	15	−5	−35
3	−3	−7	−3	17	9	21
5	1	−5	−13	−23	−5	−7
7	7	7	7	7	1	1
$\Sigma t^2 = 168$	168	264	616	2184	264	343

The problem now is to choose the levels of the dose d so that the estimates of the α's are always uncorrelated with the estimates of the β's. This problem is solved if

$$\sum t_i d^j = 0 \quad \text{for all values of } i \text{ and } j \qquad (3)$$

where the sign $\sum$ is used to denote summation over the k values of the polynomial.

*Fisher and Yates tables give these polynomials up to fifth order only, but supply a recurrence formula from which the high polynomials are readily obtained. Alternatively an extended table giving the polynomials of all orders to $k = 26$ has recently been published by De Lury (1951). A design could, of course, be developed for values of t which are not equally spaced, but in this case, the values of the orthogonal polynomials would have to be calculated. This could be done either in the straightforward manner as described for example, by Kendall (1946) or by the Choleski method of matrix inversion recently described by Rushton (1951).

Since the polynomials up to t_p are used to represent the time trend and the α's are to be uncorrelated with the β's, d must be a linear function of the extra polynomials in time of order higher than p, namely $t_{p+1}, \cdots, t_{k-1}$.

We have, therefore,

$$d = \gamma_{p+1}t_{p+1} + \cdots + \gamma_{p+r}t_{p+r} + \cdots + \gamma_{k-1}t_{k-1} \tag{4}$$

where the constants $\gamma_{p+1}, \ldots, \gamma_{k-1}$ are chosen to satisfy the relations represented by equation (3).

4.1 *Derivation of the Design Used in the Example*

To illustrate the method the design used in the example above, will be derived. In this example experience suggested that a polynomial of second degree would adequately represent the time trend whilst the log-dose/response lines would be linear. In the design it was decided to allow for the estimation of effects of one order higher than those actually expected. Allowance was made, therefore, to fit a polynomial of up to *third* degree to represent the time trend and up to *second* degree to represent the log dose/response lines.

A preliminary examination shows that to obtain a satisfactory solution and allow a reasonable number of degrees of freedom for the estimation of error at least 8 pairs of doses would be required. We have then in the notation above,

$$p = 3, \qquad q = 2, \qquad k = 8$$

Equation (4) yields

$$d = \gamma_4 t_4 + \gamma_5 t_5 + \gamma_6 t_6 + \gamma_7 t_7 \tag{5}$$

and because of the orthogonal property of the polynomials it is true whatever the values of the γ's that

$$\sum d = \sum dt_1 = \sum dt_2 = \sum dt_3 = 0. \tag{6}$$

In addition we wish to arrange that the quadratic dose effect is also orthogonal to the time effects, so that we have to satisfy the three equations

$$\sum d^2 t_1 = 0, \qquad \sum d^2 t_2 = 0, \qquad \sum d^2 t_3 = 0 \tag{7}$$

Now

$$\sum d^2 t_1 = \sum \{(\gamma_4 t_4 + \gamma_5 t_5 + \gamma_6 t_6 + \gamma_7 t_7)^2 t_1\}$$

$$= \gamma_4^2(144) + \gamma_5^2(155) + \gamma_6^2(166) + \gamma_7^2(177) + 2\gamma_4\gamma_5(145)$$

$$+ 2\gamma_4\gamma_6(146) + \cdots \text{etc.} \tag{8}$$

where for example (144) means $\sum t_1 t_4^2$. Similar expressions may be obtained for $\sum d^2 t_2$, and $\sum d^2 t_3$.

In practice most of the terms in these expanded expressions vanish since, for equally spaced values of t, the orthogonal polynomials of odd order are odd functions and those of even order are even functions. It follows that quantities like (157), (177), (144) involving three odd order polynomials or one odd and two even order polynomials are zero. Making use of the fact, the three equations derived from (7) are

$$\gamma_4\gamma_5(145) + \gamma_4\gamma_7(147) + \gamma_5\gamma_6(156) + \gamma_6\gamma_7(167) = 0 \qquad (9)$$

$$\gamma_4^2(244) + \gamma_5^2(255) + \gamma_6^2(266) + \gamma_7^2(277) + 2\gamma_4\gamma_6(246) + 2\gamma_5\gamma_7(257) = 0 \qquad (10)$$

$$\gamma_4\gamma_5(345) + \gamma_4\gamma_7(347) + \gamma_5\gamma_6(356) + \gamma_6\gamma_7(367) = 0 \qquad (11)$$

to which a wide variety of solutions may be found. In particular if d can be taken as a linear function of the *even* order polynomials only (i.e. if γ_5 and γ_7 can be put equal to zero) we shall obtain a design in which only four distinct levels of dosage are used instead of eight. Since it was of considerable practical advantage to reduce the number of dose levels, it was decided to adopt a solution of this kind.

Putting γ_5 and γ_7 equal to zero all the terms in equations (9) and (11) become zero and (10) becomes

$$\gamma_4^2(244) + 2\gamma_4\gamma_6(246) + \gamma_6^2(266) = 0 \qquad (12)$$

and the values of the constants are readily found from Table 4 of the orthogonal polynomials. In fact,

$$(244) = 160, \qquad (246) = 840, \qquad (266) = -672 \qquad (13)$$

whence writing $\gamma = \gamma_4/\gamma_6$ we have

$$160\gamma^2 + 1680\gamma - 672 = 0 \qquad (14)$$

$$\gamma = 0.385{,}823 \qquad \text{or} \qquad \gamma = -10.885{,}823 \qquad (15)$$

Hence, the levels for a design fulfilling all the requirements are provided either by

$$d = c(0.385{,}823t_4 + t_6) \text{ of by } d = c'(-10.885{,}823t_4 + t_6) \qquad (16)$$

where c and c' are scale constants at our choice. In practice it is convenient to choose the scale constant so that the 'standard deviation'

$(\sum d^2/k)^{\frac{1}{2}}$ of the levels is unity. The dose levels in these units we denote by d_1, so we have $\sum d_1^2 = k$. This choice of units enables the experimenter to assess readily the scaling-up factor which will be required so that the design covers the range of levels desired.

We require then

$$\sum d_1^2 = c^2(\gamma^2 \sum t_4^2 + \sum t_6^2) = k \tag{17}$$

whence

$$c = \left\{\frac{k}{\gamma^2 \sum t_4^2 + \sum t_6^2}\right\}^{\frac{1}{2}} \tag{18}$$

Substituting numerical values in (18) we find $c = 0.149,970,1$ $c' = 0.010,449,8$ and the required levels are

$0.057,862t_4 + 0.149,970t_6$		$-0.113,755t_4 + 0.010,450t_6$
(1) 0.555,00		(1) −0.785,84
(2) −1.502,06		(2) 1.426,57
(3) 1.176,15		(3) 0.435,31
(4) −0.229,09		(4) −1.076,05
(5) −0.229,09	or	(5) −1.076,05
(6) 1.176,15		(6) 0.435,31
(7) −1.502,06		(7) 1.426,57
(8) 0.555,00		(8) −0.785,84

In the numerical example discussed above the first of the two alternative designs was used.

In the calculation of the quadratic dose effect it is convenient to use the orthogonal quadratic polynomial which is calculated from the formula (see for example Kendall 1946)

$$d_2 = d_1^2 - (\sum d_1^3/k)d_1 - 1 \tag{19}$$

In the present example we find,

$$d_2 = d_1^2 + 0.400,748d_1 - 1 \tag{20}$$

which yields the values for the quadratic polynomial given in column (v) of Table 2.

5. DISCUSSION

From the above example the general procedure will be apparent and designs may be developed for the solution of other similar problems. As might be expected if $p + q$ is nearly as large as k no solution will be possible. On the other hand, if $p + q$ is small compared with k then a great variety of solutions may be possible.

When as in the example above a linear time trend is adequate the device of angular randomisation described by Box (1952) may be used for random selection of the design. The statistical analysis will then be exact whether the mathematical model precisely fits the situation or not.

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A DOSE-RESPONSE EQUATION FOR THE INVASION OF MICRO-ORGANISMS

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The Problem

A series of k doses (n_1 , n_2 , $\cdots$, n_k micro-organisms) is administered to m_1 , m_2 , $\cdots$, m_k test animals respectively, and the corresponding survivors are observed to number r_1 , r_2 , $\cdots$, r_k . (The terms "survivors" and "killed" whenever they occur subsequently, are meant to cover also the case when "not infected" are compared with "infected"). This paper derives a dose-response relation from a hypothesis based on the mode of action of the micro-organisms against their host first suggested by H. A. Druett (2).

Assuming that

(i) the test animals are homogeneous,
(ii) the probability of one organism killing its host is p (small),
(iii) the organisms act independently of each other,

then if n is the number of organisms administered to each subject, the expected proportion surviving is given by

$$S = (1 - p)^n \simeq e^{-np} \tag{1}$$

The statistical problem is to estimate the single parameter p from a series of observations of corresponding values of the actual proportions surviving r/m and the numbers of attacking organisms n. Equation (1) may be written

$$\ln S = -pn \tag{1a};$$

thus if we plot

$$\ln \frac{r_1}{m_1} , \ln \frac{r_2}{m_2} , \cdots , \ln \frac{r_k}{m_k}$$

against n_1 , n_2 , $\cdots$, n_k and fit a straight line to the resulting points, the negative slope of this line will represent p, the probability of any one organism killing an animal.

It follows that dose-response relationships for all kinds of organisms and test animals should be represented by a single line of constant slope when the doses are expressed as multiples of the ED_{50} . For taking c

organisms to be the ED_{50}, equation (1a) becomes

$$\ln 0.5 = -pc \tag{1b}$$

and putting $n = fc$ we obtain

$$\ln S = f \ln 0.5 \tag{2}$$

The Maximum Likelihood Solution

Let the probability that one particular animal survives be

$$P = (1 - p)^n \doteq e^{-np};$$

then the probability of r surviving out of m animals at risk will be

$$Pr\{r \text{ surv. of } m\} = \binom{m}{r} P^r (1 - P)^{m-r} \doteq \binom{m}{r} e^{-npr} (1 - e^{-np})^{m-r}$$

and the logarithm of the likelihood is

$$L = -p \sum n_i r_i + \sum (m_i - r_i) \ln (1 - e^{-n_i p}) + \text{const.}$$

Hence

$$\frac{dL}{dp} = -\sum n_i r_i + \sum (m_i - r_i) n_i \frac{e^{-n_i p}}{1 - e^{-n_i p}}$$

$$= -\sum m_i n_i + \frac{1}{p} \sum (m_i - r_i) \frac{n_i p}{1 - e^{-n_i p}} \tag{3}$$

and

$$\frac{d^2 L}{dp^2} = -\sum (m_i - r_i) n_i^2 \frac{e^{-n_i p}}{(1 - e^{-n_i p})^2}$$

$$= -\frac{1}{p^2} \sum (m_i - r_i)(n_i p)^2 \frac{e^{n_i p}}{(e^{n_i p} - 1)^2} \tag{4}$$

Substituting $n_i p = x_i$ equation (3) takes the following form for $L_{\max}$:

$$f(p) = -\sum m_i n_i + \frac{1}{p} \sum (m_i - r_i) \frac{x_i}{1 - e^{-x_i}} = 0 \tag{5}$$

and using the same substitution for equation (4) the variance of p is given by

$$\text{Var } (p) = \frac{-1}{\frac{d^2 L}{dp^2}} = \frac{p^2}{\sum (m_i - r_i) \frac{x_i^2 e^{x_i}}{(e^{x_i} - 1)^2}} \tag{6}$$

Equation (5) can be readily solved to any degree of accuracy with the help of the Table A and the application of Newton's Method of approxi-

mation; Table B evaluates expression (6) in order to obtain fiducial limits to p.

The goodness of fit can be tested with χ^2, and confidence limits can be assigned at any desired level of dose or response.

A Numerical Example

This department obtained the following data (first 4 columns of Table I) from one of a series of experiments in which *B. anthracis* spores were allowed to enter guinea pigs by the respiratory route (3).

The necessary steps to complete Table I and to obtain the required results are as follows:

(1) After having entered the values r/m in the appropriate column, ln r/m is plotted against n (preferably on log paper) and a line through the origin is fitted by eye; an estimate $p_1(0.019)$ of the slope p is thus obtained (see fig. 1).

(2) The np_1 values are computed and entered in the x-column, leaving room for corresponding values of a second cycle (np_2).

(3) Tables A and B give the following values:

x	$x/(1 - e^{-x})$	$x^2e^x/(e^x - 1)^2$
0.32	1.169	0.992
0.66	1.366	0.964
1.23	1.738	0.883
1.91	2.242	0.744

The 4 values $x/(1 - e^{-x})$ are multiplied in turn by 8, 18, 21, 28($m - r$ column) the sum of these products being 133.214; the 4 values $x^2e^x/(e^x - 1)^2$ are treated likewise yielding 64.66. Then the residue of equation (5) $f(p_1) = 80$ and Var $(p) = 0.00000558$ are obtained as set out in the lower part of Table I.

(4) A second approximation p_2 to p is computed by applying Newton's Method: $p_2 = p_1 + f(p_1)$ Var $(p) = 0.0194$.

(5) In the x-column the new pn values are entered in brackets (0.33 $\cdots$ 1.95), and $f(p_2) = +3$ is evaluated using Table A. Clearly no closer approximation is required and in fact Var (p) need not be recomputed.

(6) The expected numbers of survivors are calculated, and used to evaluate χ^2 with degrees of freedom one less than the number of doses, since one parameter has been estimated from the data.

TABLE I.

Computations for the Fitting of a Dose — ln Proportion surviving Regression Equation

n	m	r	$m-r$	r/m	x		$S = e^{-np_2}$	mS	$r - mS$	$\frac{(r-mS)^2}{mS(1-S)}$
Dose in 100 Spores	No. of animals at risk	Surviv-ing	Killed	Prop. Surv. observed	np_1	(np_2)	Prop. Surv. expected	Survivors expected		
16.8	32	24	8	0.75	0.32	(0.33)	0.72	23.0	+1.0	0.16
34.7	32	14	18	0.44	0.66	(0.67)	0.51	16.3	−2.3	0.66
64.6	32	11	21	0.34	1.23	(1.25)	0.29	9.3	+1.7	0.44
100.5	32	4	28	0.125	1.91	(1.95)	0.14	4.5	−0.5	0.06
216.6	128	53	75						$\chi^2 = 1.32$	

using equation (5):

$$-\sum mn = -216.6 \times 32 = -6931$$

$$\frac{1}{p_1}\sum (m-r)\frac{x}{1-e^{-x}} = \frac{133.214}{0.019} = 7011$$

$$f(p_1) = +80$$

$$\ln S = -0.0194n$$

$$\text{Var}(p) = \frac{0.000361}{64.66} = 0.00000558 \qquad \text{(equation 6)}$$

$$p_2 = p_1 + f(p)\ \text{Var}(p) = 0.019 + 80 \times 558 \times 10^{-8} = 0.0194$$

$$p_2' = p_2 + 1.96\sqrt{\text{Var}(p)} = 0.0240$$

$$p_2'' = p_2 - 1.96\sqrt{\text{Var}(p)} = 0.0148$$

95% fid. Lts.

ED_{50} (using equation 1b)

95% lower Lim: $\ln 0.5/(-p_2')$ $\ln 0.5/(-p_2)$ 95% upper Lim: $\ln 0.5/(-p_2'')$

28.9 ⟶ 35.7 ⟶ 46.8

TABLE A

$x/(1 - e^{-x})$

x	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
.0	1.000	1.005	1.010	1.015	1.020	1.025	1.030	1.035	1.041	1.046
.1	1.051	1.056	1.061	1.066	1.072	1.077	1.082	1.087	1.093	1.098
.2	1.103	1.109	1.114	1.119	1.125	1.130	1.136	1.141	1.147	1.152
.3	1.157	1.163	1.169	1.174	1.180	1.185	1.191	1.196	1.202	1.208
.4	1.213	1.219	1.225	1.230	1.236	1.242	1.248	1.253	1.259	1.265
.5	1.271	1.277	1.282	1.288	1.294	1.300	1.306	1.312	1.318	1.324
.6	1.330	1.336	1.342	1.348	1.354	1.360	1.366	1.372	1.378	1.384
.7	1.391	1.397	1.403	1.409	1.415	1.421	1.428	1.434	1.440	1.446
.8	1.453	1.459	1.465	1.472	1.478	1.485	1.491	1.497	1.504	1.510
.9	1.517	1.523	1.530	1.536	1.543	1.549	1.556	1.562	1.569	1.575
1.0	1.582	1.589	1.595	1.602	1.609	1.615	1.622	1.629	1.635	1.642
1.1	1.649	1.656	1.662	1.669	1.676	1.683	1.690	1.697	1.703	1.710
1.2	1.717	1.724	1.731	1.738	1.745	1.752	1.759	1.766	1.773	1.780
1.3	1.787	1.794	1.801	1.808	1.815	1.822	1.830	1.837	1.844	1.851
1.4	1.858	1.865	1.873	1.880	1.887	1.894	1.902	1.909	1.916	1.924
1.5	1.931	1.938	1.946	1.953	1.960	1.968	1.975	1.982	1.990	1.997
1.6	2.005	2.012	2.020	2.027	2.035	2.042	2.050	2.057	2.065	2.072
1.7	2.080	2.088	2.095	2.103	2.110	2.118	2.126	2.133	2.141	2.149
1.8	2.156	2.164	2.172	2.180	2.187	2.195	2.203	2.211	2.219	2.226
1.9	2.234	2.242	2.250	2.258	2.266	2.273	2.281	2.289	2.297	2.305
2.0	2 313	2.321	2.329	2.337	2.345	2.353	2.361	2.369	2.377	2.385
2.1	2.393	2.401	2.409	2.417	2.425	2.433	2.442	2.450	2.458	2.466
2.2	2.474	2.482	2.490	2.499	2.507	2.515	2.523	2.532	2.540	2.548
2.3	2.556	2.565	2.573	2.581	2.589	2.598	2.606	2.614	2.623	2.631
2.4	2.639	2.648	2.656	2.665	2.673	2.681	2.690	2.698	2.707	2.715
2.5	2.724	2.732	2.741	2.749	2.757	2.766	2.774	2.783	2.792	2.800
2.6	2.809	2.817	2.826	2.834	2.843	2.851	2.860	2 869	2.877	2.886
2.7	2.895	2.903	2.912	2.920	2.929	2.938	2.946	2.955	2.964	2.973
2.8	2.981	2.990	2.999	3.007	3.016	3.025	3.034	3.043	3.051	3.060
2.9	3.069	3.078	3.086	3.095	3.104	3.113	3.122	3.131	3.139	3.148
3.0	3.157	3.166	3.175	3.184	3.193	3.202	3.211	3.219	3.228	3.237
3.1	3.246	3.255	3.264	3.273	3.282	3.291	3.300	3.309	3.318	3.327
3.2	3.336	3.345	3.354	3.363	3.372	3.381	3.390	3.399	3.408	3.417
3.3	3.426	3.435	3.445	3.454	3.463	3.472	3.481	3.490	3.499	3.508
3.4	3.517	3.527	3.536	3.545	3.554	3.563	3.572	3.581	3.591	3.600
3.5	3.609	3.618	3.627	3.637	3.646	3.655	3.664	3.673	3.683	3.692
3.6	3.701	3.710	3.720	3.729	3.738	3.747	3.757	3.766	3.775	3.785
3.7	3.794	3.803	3.812	3.822	3.831	3.840	3.850	3.859	3.868	3.878
3.8	3.887	3.896	3.906	3.915	3.924	3.934	3.943	3.952	3.962	3.971
3.9	3.981	3.990	3.999	4.009	4.018	4.028	4.037	4.046	4.056	4.065
4.0	4.075	4.084	4.093	4.103	4.112	4.122	4.131	4.141	4.150	4.160
4.1	4.169	4.179	4.188	4.198	4.207	4.216	4.226	4.235	4.245	4.254
4.2	4.264	4.273	4.283	4.292	4.302	4.312	4.321	4.331	4.340	4.350
4.3	4.359	4.369	4.378	4.388	4.397	4.407	4.416	4.426	4.436	4.445
4.4	4.455	4.464	4.474	4.483	4.493	4.503	4.512	4.522	4.531	4.541
4.5	4.551	4.560	4.570	4.579	4.589	4.599	4.608	4.618	4.627	4.637
4.6	4.647	4.656	4.666	4.676	4.685	4.695	4.705	4.714	4.724	4.733
4.7	4.743	4.753	4.762	4.772	4.782	4.791	4.801	4.811	4.820	4.830
4.8	4.840	4.850	4.859	4.869	4.879	4.888	4.898	4.908	4.917	4.927
4.9	4.937	4.946	4.956	4.966	4.976	4.985	4.995	5.005	5.014	5.024

Abridged from "A Table of the Function $(G(x) = x/(1 - e^{-x})$ and its Applications to Problems in Compound Interest" by J. F. Steffensen, Skandinavisk Aktuarietidskrift, 1938; by kind permission of the author and the publishers.

TABLE B

$x^2e^x/(e^x - 1)^2$

x	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
.0	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.999	0.999
.1	0.999	0.999	0.999	0.999	0.998	0.998	0.998	0.998	0.997	0.997
.2	0.997	0.996	0.996	0.996	0.995	0.995	0.994	0.994	0.993	0.993
.3	0.993	0.992	0.992	0.991	0.990	0.990	0.989	0.989	0.988	0,987
.4	0.987	0.986	0.985	0.985	0.984	0.983	0.983	0.982	0.981	0.980
.5	0.979	0.979	0.978	0.977	0.976	0.975	0.974	0.973	0.972	0.971
6	0.971	0.970	0.969	0.968	0.967	0.966	0.964	0.963	0.962	0.961
.7	0.960	0.959	0.958	0.957	0.956	0.954	0.953	0.952	0.951	0.950
.8	0.948	0.947	0.946	0.945	0.943	0.942	0.941	0.939	0.938	0.937
.9	0.935	0.934	0.932	0.931	0.930	0.928	0.927	0.925	0.924	0.922
1.0	0.921	0.919	0.918	0.916	0.915	0.913	0.911	0.910	0.908	0.907
1.1	0.905	0.903	0.902	0.900	0.898	0.897	0.895	0.893	0.892	0.890
1.2	0.888	0.886	0.885	0.883	0.881	0.879	0.878	0.876	0.874	0.872
1.3	0.870	0.868	0.867	0.865	0.863	0.861	0.859	0.857	0.855	0.853
1.4	0.852	0.850	0.848	0.846	0.844	0.842	0.840	0.838	0.836	0.834
1.5	0.832	0.830	0.828	0.826	0.824	0.822	0.820	0.818	0.816	0.814
1.6	0.811	0.809	0.807	0.805	0.803	0.801	0.799	0.797	0.795	0.792
1.7	0.790	0.788	0.786	0.784	0.782	0.780	0.777	0.775	0.773	0.771
1.8	0.769	0.767	0.764	0.762	0.760	0.758	0.755	0.753	0.751	0.749
1.9	0.747	0.744	0.742	0.740	0.738	0.735	0.733	0.731	0.729	0.726
2.0	0 724	0.722	0.720	0.717	0.715	0.713	0.710	0.708	0.706	0.704
2.1	0.701	0.699	0.697	0.694	0.692	0.690	0.687	0.685	0.683	0.681
2.2	0.678	0.676	0.674	0.671	0.669	0.667	0.664	0.662	0.660	0.657
2.3	0.655	0.653	0.651	0.648	0.646	0.644	0.641	0.639	0.637	0.634
2.4	0.632	0.630	0.627	0.625	0.623	0.620	0.618	0.616	0.614	0.611
2.5	0.609	0.607	0.604	0.602	0.600	0.597	0.595	0.593	0.590	0.588
2.6	0.586	0.584	0.581	0.579	0.577	0.574	0.572	0.570	0.568	0.565
2.7	0.563	0.561	0.559	0.556	0.554	0.552	0.549	0.547	0.545	0.543
2.8	0.540	0.538	0.536	0.534	0.532	0.529	0.527	0.525	0.523	0.520
2.9	0.518	0.516	0.514	0.512	0.509	0.507	0.505	0.503	0.501	0.498
3.0	0.496	0.494	0.492	0.490	0.488	0.485	0.483	0.481	0.479	0.477
3.1	0.475	0.473	0.470	0.468	0.466	0.464	0.462	0.460	0.458	0.456
3.2	0.454	0.452	0.449	0.447	0.445	0.443	0.441	0.439	0.437	0.435
3.3	0.433	0.431	0.429	0.427	0.425	0.423	0.421	0.419	0.417	0.415
3.4	0.413	0.411	0.409	0.407	0.405	0.403	0.401	0.399	0.397	0.395
3.5	0.393	0.391	0.389	0.388	0.386	0.384	0.382	0.380	0.378	0.376
3.6	0.374	0.372	0.371	0.369	0.367	0.365	0.363	0.361	0.359	0.358
3.7	0.356	0.354	0.352	0.350	0.349	0.347	0.345	0.343	0.342	0.340
3.8	0.338	0.336	0.334	0.333	0.331	0.329	0.328	0.326	0.324	0.322
3.9	0.321	0.319	0.317	0.316	0.314	0.312	0.311	0.309	0.307	0.306
4.0	0.304	0.302	0.301	0.299	0.298	0.296	0.294	0.293	0.291	0.290
4.1	0.288	0.286	0.285	0.283	0.282	0.280	0.279	0.277	0.276	0.274
4.2	0.273	0.271	0.270	0.268	0.267	0.265	0.264	0.262	0.261	0.259
4.3	0.258	0.256	0.255	0.254	0.252	0.251	0.249	0.248	0.246	0.245
4.4	0.244	0.242	0.241	0.239	0.238	0.237	0.235	0.234	0.233	0.231
4.5	0.230	0.229	0.227	0.226	0.225	0.223	0.222	0.221	0.220	0.218
4.6	0.217	0.216	0.215	0.213	0.212	0.211	0.210	0.208	0.207	0.206
4.7	0.205	0.203	0.202	0.201	0.200	0.199	0.197	0.196	0.195	0.194
4.8	0.193	0.192	0.190	0.189	0.188	0.187	0.186	0.185	0.184	0.183
4.9	0.181	0.180	0.179	0.178	0.177	0.176	0.175	0.174	0.173	0.172

Abridged from "An 8 place Table of the Einstein Functions" by J. Sherman and R. B. Ewell, The Journal of Physical Chemistry, June 1942 by kind permission of The Williams & Wilkins Company, Baltimore.

Experimental Verification of the Hypothesis

The extent to which the basic hypothesis fits the experimental facts is shown by the figures in Table II which gives the pooled values of χ^2 for several experiments on these different micro-organisms. The individual responses are plotted in figs. 2a, b, c; the doses have been expressed as multiples of the ED_{50}'s, and the lines have the theoretical slope of ln 0.5. It should be noted that in the case of *B. anthracis* the experiments were not replicates since the particle size of the cloud was made to vary considerably from experiment to experiment, so that individual regres-

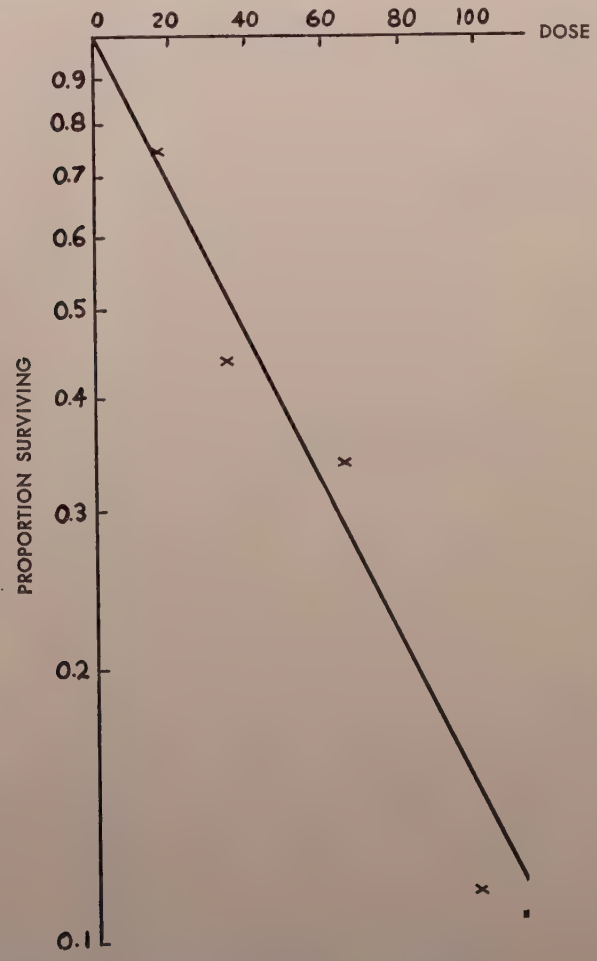

FIG. 1.

Relationship between dose of *B. anthracis* spores and ln proportion surviving of guinea pigs, showing regression line (fitted by eye).

sion lines were obtained in widely different positions. The 8 experiments on *B. typhosum* were picked at random from 56 similar ones.

TABLE II. VARIOUS RELATIONSHIPS FITTING ONE FIXED LINE

Organism	Test Animals	Route of Infection	No. of Expts.	χ^2	Degr. of fr.	Graph
Brucella suis (4)	300 Guinea pigs	Respiratory	4	3.2	6	fig. 2a
B. anthracis (3)	990 Guinea pigs	Respiratory	5	25.9	22	fig. 2b
B. typhosum (1)	640 Mice	Intraperitoneally	8	19.8	14	fig. 2c

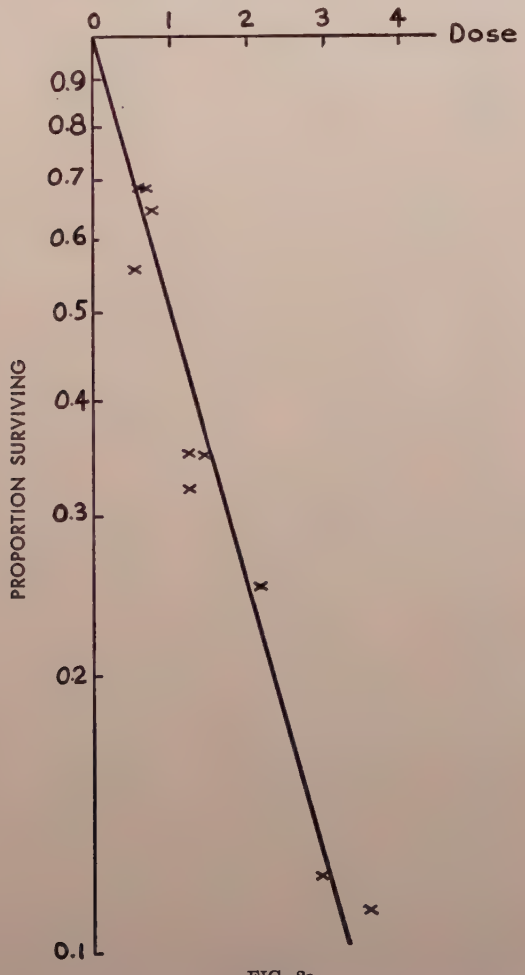

FIG. 2a.

Fixed regression line in relation to the result of 4 experiments with *Brucella suis*; doses of the sub groups are expressed in multiples f of the ED_{50} of each individual experiment.

Estimation of Relative Potency

If different strains of the same pathogenic organism are to be compared as to their virulence with respect to a host, one dose-response line is fitted for each strain and the ratio of the slopes estimates the relative potency. For let p_s and p_u be the slopes of "standard" and "unknown" respectively and n_s and n_u be doses producing a common response S; it follows from equation (1a) that $n_s p_s = n_u p_u$ or

$$\text{Relative Potency } R = \frac{n_s}{n_u} = \frac{p_u}{p_s}$$

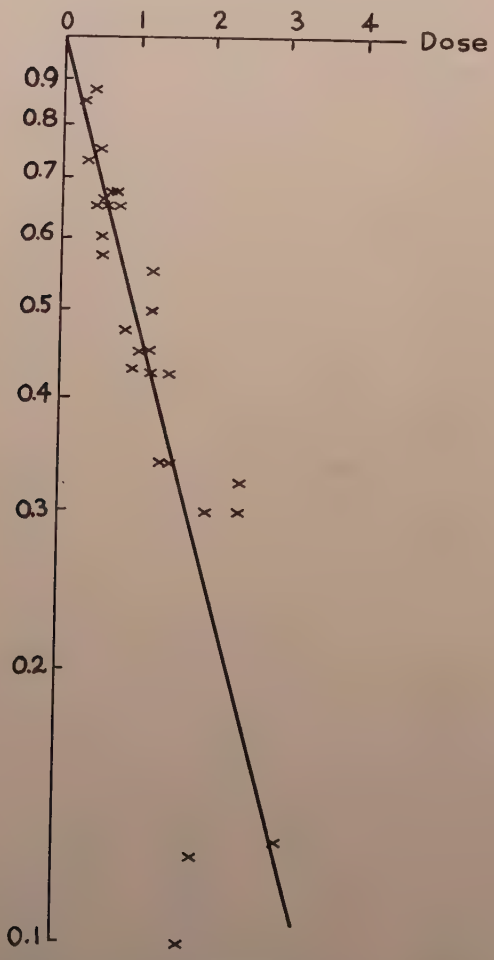

FIG. 2b.

Fixed regression line in relation to the result of 5 experiments with *B. anthracis*

Since Var (p_u) and Var (p_s) have been computed already together with p_u and p_s, fiducial limits to R are readily assigned as

$$\frac{R \pm \sqrt{g[R^2 + (1 - g)\ \text{Var}\ p_u/\text{Var}\ p_s]}}{1 - g}$$

where $g = t^2\ \text{Var}\ p_s/p_s^2$ and t is the normal deviate for the level of probability to be used (5).

Comparison with probit analysis

This Department used to apply the methods of probit analysis to microbiological dose-response relationships (6). When for example ani-

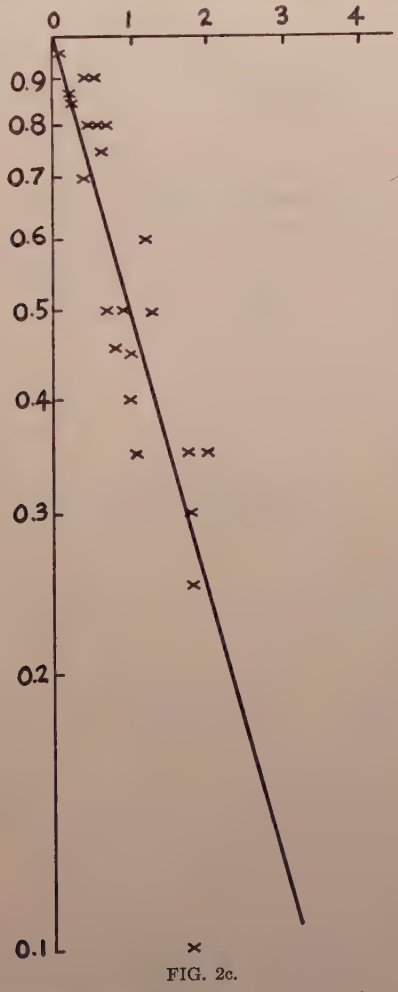

FIG. 2c.

Fitted regression line in relation to the results of 8 experiments with *B. Typhosum.*

mals were exposed to pathogens, a ($\log_{10}$ dose) − (probit killed) regression equation has fitted well the results in most cases. If the hypothesis expressed in this paper holds good, i.e. if $\ln S = -pn$ (equation 1a) actually applies, all the probit lines computed so far ought to show about the same slope within experimental error (Examples are quoted in Ref. 2). For $\log_{10}$ dose − probit killed transforms an ideal dose − ln proportion surviving into a slightly bent curve (see fig. 3) and it is easily proved that, if Y is the Probit, the slope $dY/d \log_{10} n$ at the ED_{50} equals 2 (very nearly)

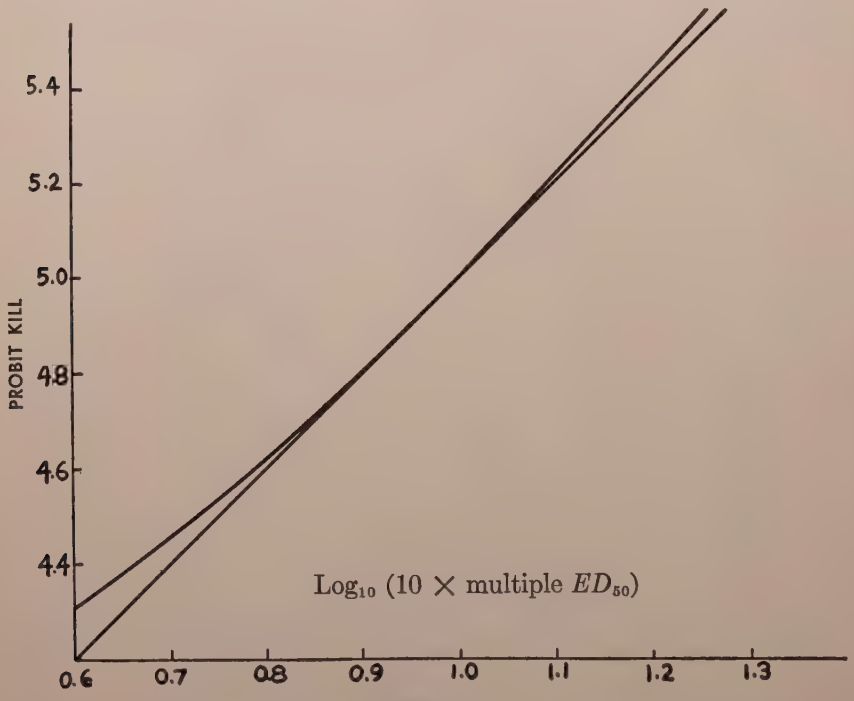

FIG. 3.

An ideal dose − ln proportion surviving relationship plotted as probits against log dose. The slope at the ED_{50} is 2.

Consider

$$1 - S = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{Y-5} e^{-\frac{1}{2}u^2}\, du \qquad \text{and} \qquad \ln S = -pn$$

Now

$$\frac{dY}{dS} = -\sqrt{2\pi}\, e^{\frac{1}{2}(Y-5)^2} \qquad \text{(A)}$$

$$\frac{dS}{dn} = -Sp \qquad \text{(B) and}$$

$$\frac{dn}{d \log_{10} n} = n \log_e 10 \qquad \text{(C);}$$

Substituting in the product of (A) × (B) × (C) for $Y = 5$, for $S = 0.5$ and for $pn = -\ln 0.5$ gives 2.0003.

It may be concluded that

(i) the two models differ only slightly and could not be experimentally distinguished without forbidding expenditure in test animals,
(ii) that the calculated ED_{50} should be on the whole lower when probit analysis is used (see Table III),
(iii) the present method compares favourably with probit analysis as far as computation labour is concerned.

Examples from practice have been worked out by both methods and the results are given in Tables III and IIIA. The estimates of relative potencies provided by the two techniques are in good agreement throughout; the probit method appears in general to give slightly narrower fiducial belts but the point at issue, of course, is not which method gives more apparent precision but which one is based on the more appropriate mathematical model.

TABLE III. COMPARISON OF TWO METHODS OF ANALYSIS

A.....probit analysis
B.....present method

Organism	χ^2/df.		ED_{50} and its 95% fid. lts.	
	A	B	A	B
B.anthracis, 1μ particles*	0.72	0.44	0.24 → 0.34 → 0.43	0.29 → 0.36 → 0.47
B.anthracis, 3.5μ particles	1.33	0.94	0.29 → 0.36 → 0.44	0.30 → 0.36 → 0.47
B.anthracis, 4.5μ particles	1.16	1.08	0.40 → 0.51 → 0.70	0.44 → 0.53 → 0.68
B.anthracis, 8μ particles	1.46	1.51	3.3 → 3.8 → 4.7	3.3 → 4.0 → 5.1
B.anthracis, 12μ particles	0.10	1.28	4.0 → 5.3 → 7.1	5.1 → 6.0 → 7.3
Brucella suis	0.06	0.77	6.9 → 31.7 → 52.7	34.0 → 45.3 → 68.0
Brucella suis	0.88	0.67	25.3 → 35.3 → 57.0	25.5 → 35.0 → 55.9
Brucella suis	0.26	0.05	21.0 → 34.8 → 47.9	26.0 → 36.1 → 57.0
Brucella suis	0.44	0.41	19.0 → 31.8 → 44.1	24.1 → 32.7 → 51.0

*See Table I.

TABLE IIIA. POTENCIES OF 2 MUTANTS OF Ty_{22}—STRAIN OF *B. Typhosum* RELATIVE TO PARENT STRAIN. 2 DIFFERENT EXPERIMENTS. RESULTS OBTAINED BY THE TWO METHODS

Expt.	Organism	χ^2/df.		Rel. Potency and its 95% fid. lts.	
		A	B	A	B
MVT. 75	Ty_{22}	1.96	3.04		
	Mutant a	0.27	0.23	2.34 →3.39 →4.90	2.17 →3.43 →5.78
	Mutant b	0.19	1.60	0.73 →1.05 →1.50	0.58 →1.01 →1.70
MVT. 85	Ty_{22}	0.95	0.56		
	Mutant a	0.24	1.27	0.02 →0.029→0.042	0.019→0.030→0.049
	Mutant b	0.27	1.47	0.047→0.069→0.099	0.057→0.075→0.110

Choice of doses

When experimental evidence has shown that the dose — ln proportion surviving regression line holds good for a certain host-parasite relationship, the question arises: What is the most economical investment of test animals? In other words: Which dose n will minimise the variance of the regression coefficient p?

Using equation (4) for one point:

$$\frac{d^2L}{dp^2} = -(m - r)n^2 \frac{e^{pn}}{(e^{pn} - 1)^2}$$

and putting $(m - r)$ equal to its expected value $m(1 - e^{-pn})$ we obtain

$$\frac{-1}{\frac{d^2L}{dp^2}} = \text{Var}\,(p) = \frac{e^{pn} - 1}{mn^2} \tag{7}$$

and hence the Invariance of p can be expressed in the form

$$\frac{1}{\text{Var}\,(p)} = \frac{m}{p^2} \frac{(\ln S)^2\, S}{1 - S} \tag{7a}$$

Differentiating equation (7) w.r. to n and putting

$$\frac{d(\text{Var}\,p)}{dn} = 0,$$

the equation is simplified to

$$e^{pn}(2 - pn) - 2 = 0. \tag{8}$$

Hence Var (p) is a minimum for

$$pn = 1.5936 \tag{9}$$

which corresponds to

$$e^{-1.59} = 20.3\% \text{ survivors} \tag{9a}$$

$$\text{Inv } (p)_{\max} = \frac{m}{p^2} \times 0.6476 \tag{9b}$$

In fig. 4 equation (7a) is plotted as a percentage of its maximum value (equation 9b). It will be noted that the efficiency within the range of 10%–35% survivors is hardly affected; in practice the experimentor would aim at a moderately low survivor rate and know that, if he misses within reason the theoretical 20.3% optimum (equation 9a), economy would still not be appreciably impaired.

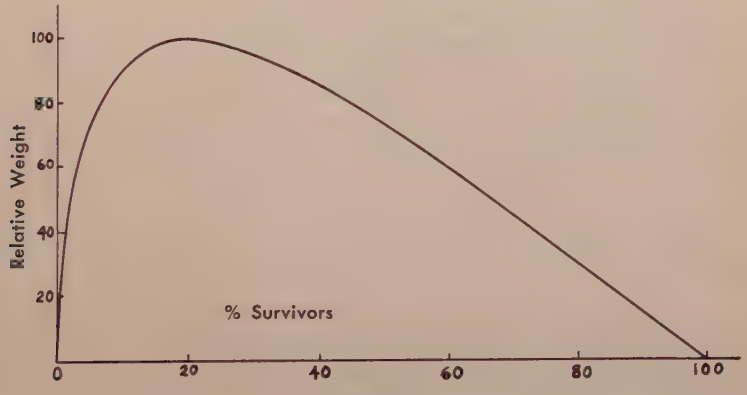

FIG. 4.

Relative Invariance of an estimate of p based on a single dose-level as a function of the proportion surviving.

If little is known about p and even linearity is doubtful, obviously more than one point is needed; but high survivor rates which carry little weight will be avoided, if possible.

Application to dilution series

After this paper had been drafted the close analogy with the analysis of dilution series was pointed out to me by Mr. M. J. R. Healy. Using the notation of this paper the relevant problem can be presented in the following rather condensed form. (For details references (7), (8) may be consulted.)

A series of k dilutions with dilution factors $n_1 , n_2 , \cdots , n_k$ is prepared from a bacterial suspension whose density of p organisms per unit volume is to be estimated, $m_1 , m_2 , \cdots , m_k$ samples respectively are incubated and the resulting sterile samples are observed to number $r_1 , r_2 , \cdots , r_k$ respectively. It can be shown that under certain assumptions the expected proportion of sterile samples $S = e^{-pn}$ (7).

Obviously the present method should be able to deal with the problem. Fisher and Yates (8) give an example (the tests of a potato flour for the estimation of the density of spores of *B. mesentericus*) to illustrate Fisher's approximative method which Finney also used to elucidate his method (7).

Table IV gives the estimated density of organisms per gram with its 95% limits by the three techniques.

TABLE IV.

	Density (organisms/gram)	95% limits
Fisher	760	407–1440
Finney	766	429–1370
Present method	766	321–1211

Summary

The mode of action of pathogenic micro-organisms is explained by a hypothesis (first given by H. A. Druett) which leads to a linear relation between dose and log proportion surviving; its maximum likelihood solution is given and tables are provided to help computation. When doses are expressed in terms of the ED_{50} a fixed regression line to fit all pathogenic relationships emerges from the underlying hypothesis. Illustrative examples taken from practice are quoted. Comparison with Probit analysis is discussed. The problem of economical use of test animals is treated. The method can be applied to dilution series.

Acknowledgements

It is a pleasure to thank Mr. E. C. Fieller, Ministry of Supply, for encouragement and helpful suggestions while this paper was being prepared. I am indebted to Mr. R. Ash for helping with the necessary computations. Acknowledgement is made to the Chief Scientist, Ministry of Supply, for permission to publish this paper.

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Correction to "II. Some Statistical Problems in Measuring the Subjective Response to Drugs" by Frederick Mosteller, *Biometrics*, Vol. 8, No. 3, 1952, p. 220–231. In this article McNemar's formula for testing for changes in a 2×2 table is discussed. The reader was referred to Cochran's article (The comparison of percentages in matched samples, *Biometrika*, Vol. 37 (1950), pp. 256–266) for a discussion of related and more complicated problems. However, it was erroneously stated that Cochran's description of the test does not contain the correction term. In the text immediately following the formula, Cochran gives the method for correcting for continuity.

Errata: "Design and Analysis of Triangular Singly Linked Blocks" by K. R. Nair. *Biometrics*, Vol. 9, No. 2, 1953, p. 147. In the paragraph just below Table 3, "inter-block" should be changed to "intra-block."

Errata: Abstract 231, *Biometrics*, Vol. 9, No. 2, 1953, p. 267. In the title of the abstract by J. A. Fraser Roberts, the word "Independent" should be changed to "Dependent."

ANALYSIS OF EGG SHAPE OF CHICKENS

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The principles of population genetics as formulated by Wright (1921) elucidated by Lush (1945), and applied to poultry by Lerner (1950), provide the basis for an investigation into the relative contributions of heredity and environment to egg shape. The purpose of this study is twofold: (1) from the practical standpoint, a knowledge of the heritability of egg shape and the correlation of egg shape with characters under selection, particularly egg weight, may be useful in breeding programs; (2) from the theoretical (and ultimately practical) standpoint, egg shape is a convenient character for studies on the relationship of environmental factors to balanced genetic systems.*

The definition of egg shape used here is 100 times the maximum breadth divided by the greatest length. This shape index was proposed by Pearl and Surface (1914) and is the one used by Marble (1943) who postulated the polygenic nature of this trait.

MATERIALS

The populations used in this study consist of the production-bred flock (Lerner, 1950) and eight single sire inbred lines (Shultz, 1953) maintained at the University of California. Measurements were obtained from 498 pullets produced from 12 sires and 117 dams of the production-bred flock. The eight inbred lines were derived from the production-bred flock in 1944. Two lines each were selected for high (*HNP*, *HNM*) and low (*LNP*, *LNM*) November egg number and high (*HWP*, *HWM*) and low (*LWP*, *LWM*) November egg weight. Data from reciprocal crosses between lines selected similarly (e.g. *HNP* $\times$ *HNM*) are also analyzed. Measurements were obtained for a total of 134 pullets from these lines and 154 pullets from the crosses between lines. All pullets studied were hatched in 1950.

The first and second egg from each of two clutches from each pullet were measured during March and April, 1951. Double-yolked eggs, wind eggs, rough shelled eggs, and abnormally shaped eggs were not considered. Measurements of length and breadth were made with

*This paper is one of a group entitled *Inbreeding and balanced genetic systems in the chicken* on file as a Ph.D. thesis in the library of the University of California, Berkeley. For other published material from this thesis, see Shultz and Briles (1953) and Shultz (1953).

micrometers, readings being taken to the nearest hundredth of a millimeter.

PRODUCTION-BRED FLOCK

Variance analysis of positions combined. The variance components to be considered are S (between sires), D (between dams within sires), P (between pullets within dams), U (between clutches within pullets), Q (between the two positions of a clutch), and I_{QS} , I_{QD} , I_{QP} , and I_{QU} (interactions of positions with sires, with dams within sires, with pullets within dams, and with clutches within pullets, respectively). The component I_{QU} should more properly be considered an error term. The two forms of analysis of variance used for computations and their relationship to the third form of partition of variance from which the various components of mean squares can be obtained are shown in figur e 1

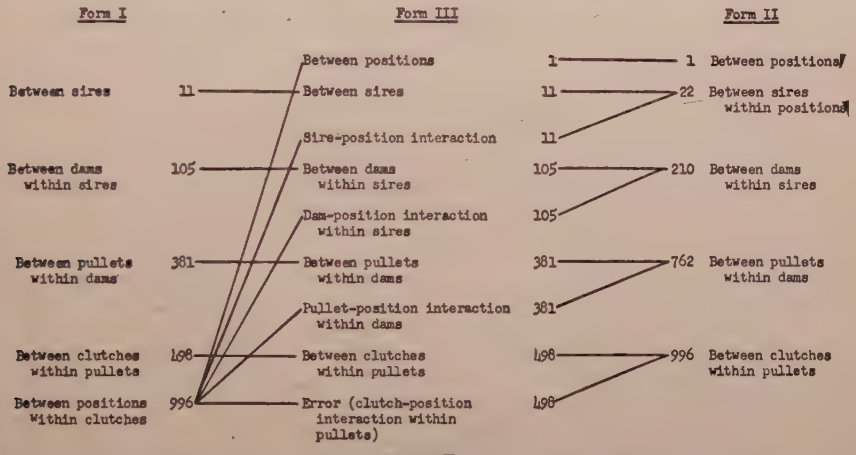

FIGURE 1.
RELATIONSHIPS BETWEEN FORMS OF ANALYSIS OF VARIANCE. FORM III IS DERIVED BY SUBTRACTIONS OF SUMS OF SQUARES OF FORM I FROM FORM II. NUMBERS REFER TO DEGREES OF FREEDOM.

Since there are equal numbers of eggs from each clutch position within each of the other subclases (sire, dam, or pullet) unbiased estimates of the interaction terms between positions and the other sources of variation can be obtained.

The mean squares of Form III of the analysis of variance, their composition in terms of components, and the tests of significance of the various interactions are presented in table 1. The composition of mean squares indicates which comparisons should be made for the F test. For example, the sire-position interaction mean square should be compared to the dam-position interaction in the test of significance of I_{QS} .

TABLE 1

Analysis of variance (including both clutch positions) in the production-bred flock.

Source of Variation (Form III in fig. 1)	Composition of mean square	Degrees of freedom	Mean square	F	Component test
B. sires	$I_{QU} + \bar{n}_u U + \bar{n}_p P + \bar{n}_{qp} I_{QP} + \bar{n}_d D + \bar{n}_{qd} I_{QD} + \bar{n}_s S + \bar{n}_{qs} I_{QS}$	11	172.91		
B. dams w. sires	$I_{QU} + \bar{n}_u U + \bar{n}_p P + \bar{n}_{qp} I_{QP} + \bar{n}_d D + \bar{n}_{qd} I_{QD}$	105	25.84		
B. pullets w. dams w. sires	$I_{QU} + \bar{n}_u U + \bar{n}_p P + \bar{n}_{qp} I_{QP}$	381	29.48		
B. positions	$I_{QU} + \bar{n}_{qp} I_{QP} + \bar{n}_{qd} I_{QD} + \bar{n}_{qs} I_{QS} + \bar{n}_q Q$	1	45.00	11.51*	Q
Sire-position interaction	$I_{QU} + \bar{n}_{qp} I_{QP} + \bar{n}_{qd} I_{QD} + \bar{n}_{qs} I_{QS}$	11	3.91	1.01	I_{QS}
Dam-position interaction w. sires	$I_{QU} + \bar{n}_{qp} I_{QP} + \bar{n}_{qd} I_{QD}$	105	3.89	1.21	I_{QD}
Pullet-position interaction w. dams w. sires	$I_{QU} + \bar{n}_{qp} I_{QP}$	381	3.21	1.01	I_{QP}
B. Clutches w. pullets w. dams w. sires	$I_{QU} + \bar{n}_u U$	498	4.40	1.39*	U
Clutch-position interaction w. pullets w. dams w. sires	I_{QU}	498	3.17		
	Combined form				
B. positions	Error $+ \bar{n}_q Q$	1	45.00	13.76*	Q
B. clutches	Error $+ \bar{n}_u U$	498	4.40	1.35*	U
Error (all interaction terms)	Error	995	3.27		

*Significant at 1% level

None of the interaction terms are significant nor do their relative magnitudes suggest that there is any interaction present.

In the absence of interaction, all interaction terms may be combined to give a new error term to be used in the test of significance of the between positions and between clutches mean squares (table 1). A significant difference (.01 level) between positions and between clutches was found.

The mean of the first position (73.55) was significantly lower than the mean of the second position (73.87). This is in agreement with the data of Marble (1943) in which the first egg of the clutch was almost always longer and narrower than subsequent eggs in the same clutch.

The significant difference between clutches suggests a seasonal trend in egg shape. This was found to be the case, the mean index of second clutch eggs (73.96) being higher than that of first clutch eggs (73.48). The direction of the difference was consistent for all 12 of the sire families taken separately. Lerner (unpublished) found a significant difference between eggs laid in the fall and eggs laid in the spring in a group of 24 SCW Leghorn pullets from a different flock. Benjamin (1920) also found a significant seasonal trend in SCWL chickens, the index reaching a peak in the fifth month of production and then declining. Marble (1943), on the other hand, failed to find any indications of a seasonal trend in a flock of Barred Plymouth Rocks.

The heritability of the shape index on the basis of a single egg per pullet, assuming that the egg was selected at random from among the four eggs measured, was estimated at .124 (table 5). The heritability on the basis of four eggs per pullet as measured here and on the theoretical basis of an infinite number of first and second position eggs was estimated at .167 and .189 respectively.

Variance analysis of positions separately. The lack of interactions between positions and other sources of variance allows considerable simplification in the analysis of variance. Considering each position separately, the form of the analysis of variance. and the composition of mean squares becomes that shown in table 2. The interpretations of these components are given in table 3 in which $\sigma^2_{G_A}$ is the genetic variance due to autosomal genes, $\sigma^2_{G_{SL}}$ is the genetic variance (in the heterogametic females) due to sex-linked genes, σ^2_C is the variance due to maternal effects, σ^2_E is the variance due to environmental effects common to all eggs of the same position of an individual bird, $\sigma^2_{\bar{E}}$ is the variance due to environmental causes peculiar to each egg, and $r^{G_A}_{f_s}$, $r^{G_{SL}}_{f_s}$, $r^{G_A}_{hs}$, $r^{G_{SL}}_{hs}$ are the autosomal and sex-linked genetic correlations for full and half-sibs. The values of the variance components are presented in table 4.

The analytical design (table 3) will not allow the disentangling of all

TABLE 2

Form of analysis of variance for clutch-positions separately and the covariance between clutch-positions

Source of variation	df.	Composition of mean square (or covariance)
Between sires	11	$U + \bar{n}_p P + \bar{n}_d D + \bar{n}_s S$
Between dams within sires	105	$U + \bar{n}_p P + \bar{n}_d D$
Between pullets within dams	381	$U + \bar{n}_p P$
Between clutches within pullets	498	U

of the various genetic and environmental variances. Although the effects due to sex-linkage would be expected to be small (see discussion by Lerner, 1950) the sire component exceeded the dam component for each of the two positions suggesting that some sex-linkage does exist and that maternal effects are small or absent. Consequently, the best estimate of the total genetic variance appears to be twice the sum of the sire and dam components or $2(S + L)$. The enrivonmental variance designated σ_E^2 can best be estimated by the difference between the pullet component and the sum of the sire and dam components or $P - (S + D)$. The estimate of the genetic variance will contain twice the maternal effect whereas the estimate of σ_E^2 will be too small by the magnitude of the maternal effect. Since the maternal effect is small if present at all, estimates obtained in the above manner should not be in serious error from this cause.

The relationships between the various causes of variation are shown by the path diagram for one position in figure 2. The genotypes of the sire and dam (G_S and G_D respectively) each contribute to the genotype of the pullet (G_P), r_{fs}^G and r_{hs}^G being the correlations between full and half-sibs respectively. The genotype of a pullet and the environment (E) contribute to what may be called the potential capacity of the individual to produce eggs of a particular shape (K). In turn, this constitution and additional environmental factors ($\hat{E}$) determine the final shape of the egg (Z). The $\hat{E}$ portion of the environment represents factors varying in their effects from time to time during the period of lay including errors of measurement. The phenotypic correlations between eggs are designated by t. If more than one egg of the same position from the same pullet is measured (in this case 2), the shape of each egg will contribute to a mean phenotype ($\bar{Z}$) for that bird.

Heritability is the ratio of the additive genetic variation to the total variation. Hence, a decrease in the environmental portion of the total

TABLE 3

Interpretations of components of variance and covariance analyses. Substitutions in general form made for females only. F is Wrights (1921) coefficient of inbreeding of the generation analysed. F′ is the coefficient of inbreeding of the parents. Formulas for genetic correlations under inbreeding (including those for males) are also given. For single sire lines, lines and sires become synonymous and $r^{G}{}_{s}$ equals zero.

A—autosomal. *SL*—sex-linked. F_A does not equal F_{SL}.

Component	General form*	Non-inbred population	
		Variance	Covariance
L	$r_e^{G_A}(1+F_A)\sigma^2_{G_A} + r_e^{G_{SL}}(1+F_{SL})\sigma^2 G_{SL}$	—	—
S	$(r_{hs}^{G_A} - r_e^{G_A})(1+F_A)\sigma^2_{G_A}$ $+ (r_{hs}^{G_{SL}} - r_e^{G_{SL}})(1+F_{SL})\sigma^2_{G_{SL}}$	$\frac{1}{4}\sigma^2_{G_A} + \frac{1}{2}\sigma^2_{G_{SL}}$	$\frac{1}{4}COV_{G_A} + \frac{1}{2}COV_{G_{SL}}$
D	$(r_{fs}^{G_A} - r_{hs}^{G_A})(1+F_A)\sigma^2_{G_A}$ $+ (r_{fs}^{G_{SL}} - r_{hs}^{G_{SL}})(1+F_{SL})\sigma_{G_{SL}} + \sigma^2_C$	$\frac{1}{4}\sigma^2_{G_A} + \sigma^2_C$	$\frac{1}{4}COV_{G_A} + COV_C$
P	$(1 - r_{fs}^{G_A})(1+F_A)\sigma^2_{G_A}$ $+ (1 - r_{fs}^{G_{SL}})(1+F_{SL})\sigma^2_{G_{SL}} + \sigma^2_E$	$\frac{1}{2}\sigma^2_{G_A} + \frac{1}{2}\sigma^2_{G_{SL}} + \sigma^2_E$	$\frac{1}{2}COV_{G_A} + \frac{1}{2}COV_{G_{SL}} + COV_E$
U	σ^2_E	σ^2_E	COV_E
Total	$(1+F_A)\sigma^2_{G_A}$ $+ (1+F_{SL})\sigma^2_{G_{SL}} + \sigma^2_C + \sigma^2_E + \sigma^2_E$	$\sigma^2_{G_A} + \sigma^2_{G_{SL}} + \sigma^2_C + \sigma^2_E + \sigma^2_E$	$COV_{G_A} + COV_{G_{SL}} + COV_C$ $+ COV_E + COV_E$

		Inbred lines	
Autosomal, males. and females:	$r_e^{G_A} = \frac{2F_A}{1+F_A}$,	$r_{hs}^{G_A} = \frac{1+F'_A+6F_A}{4(1+F_A)}$	$r_{fs}^{G_A} = \frac{1+F'_A+2F_A}{2(1+F_A)}$
Sex-linked, females**:	$r_e^{G_{SL}} = F_{SL}$,	$r_{hs}^{G_{SL}} = \frac{1+F'_{SL}}{2}$	$r_{fs}^{G_{SL}} = \frac{1+F'_{SL}}{2}$
Sex-linked, males***:	$r_e^{G_{SL}} = \frac{2F_{SL}}{1+F_{SL}}$,	$r_{hs}^{G_{SL}} = \frac{1+F'_{SL}+6F_{SL}}{4(1+F_{SL})}$	$r_{fs}^{G_{SL}} = \frac{3+F'_{SL}+4F_{SL}}{4(1+F_{SL})}$
		Single sire line crosses	
Autosomal, males, and females:	$r_e^{G_A} = \frac{2F_A}{1+F_A}$,	$r_{hs}^{G_A} = \frac{1+F'_A+2F_A}{4}$	$r_{hs}^{G_A} = \frac{1+F'_A}{2}$
Sex-linked, females***:	$r_e^{G_{SL}} = F_{SL}$,	$r_{hs}^{G_{SL}} = \frac{1+F'_{SL}}{2}$	$r_{fs}^{G_{SL}} = \frac{1+F'_{SL}}{2}$
Sex-linked, males***:	$r_e^{G_{SL}} = \frac{2F_{SL}}{1+F_{SL}}$,	$r_{hs}^{G_{SL}} = \frac{1+F'_{SL}+2F_{SL}}{4}$	$r_{fs}^{G_{SL}} = \frac{3+F'_{SL}}{4}$

*Covariance follows this same form.

**For sex linkage, the genetic variance as measured in the heterogametic sex is not increased by inbreeding. Therefore the term $(1+F_{SL})$ given in the general formula becomes 1 in the case of females.

***Correlations for non-inbred populations as they apply to poultry are given by Lerner (1950, table 9). However, a mistake was made in the derivation of Lerner's table from that given by Lush (1945) for mammals. The correct values for the last three lines in Lerner's table dealing with paternal half sibs relationships should read 0.50, 0.25, and 0.35 for the case in which all genes are sex-linked and 268, .250, and .256 for the case in which five per cent of the genes are sex-linked.

TABLE 3—*Continued*

Component	Single sire inbred lines** (Genetic variance only)	Line crosses (Genetic variance only)
L	—	—
S	$\frac{1+F'_A+6F_A}{4}\sigma^2_{G_A}+\frac{1+F'_{SL}}{2}\sigma^2_{G_{SL}}$	$\frac{1+F'_A+2F_A}{4}\sigma^2_{G_A}+\frac{1+F'_{SL}}{2}\sigma^2_{G_{SL}}$
D	$\frac{1+F'_A-2F_A}{4}\sigma^2_{G_A}$	$\frac{1+F'_A-2F_A}{4}\sigma^2_{G_A}$
P	$\frac{1-F'_A}{2}\sigma^2_{G_A}+\frac{1-F'_{SL}}{2}\sigma^2_{G_{SL}}$	$\frac{1-F'_A}{2}\sigma^2_{G_A}+\frac{1-F'_{SL}}{2}\sigma^2_{G_{SL}}$
U	—	—
Total	$(1+F_A)\sigma^2_{G_A}+\sigma^2_{G_{SL}}$	$\sigma^2_{G_A}+\sigma^2_{G_{SL}}$

variance will result in an increase in the heritability. Such a decrease in the effects of the daily environment (E) can be obtained by taking more than one measurement of egg shape of a pullet. This leads to the possibility of three estimates of heritability:

h^2_z pertains to the single egg. This determination, in which all of the effects of the transient environment ($\hat{E}$) are present, is analogous to estimates of characters for which only one measurement is possible or commonly taken (e.g. egg number, body size).

$h^2_{\bar{z}}$ depends on the number (n) of eggs measured per pullet. The greater the number of eggs measured the higher will be this estimate assuming that the components of variance remain constant over the longer period of time required to obtain the additional measurements, an assumption not necessarily warranted. This estimate is analogous to those for egg weight, several eggs from each bird commonly being weighed.

h^2 constituted the limiting case in which n is assumed to be infinity.

The estimates of the various heritabilities and environmental effects are given in table 5.

Covariance analysis. Because a significant difference between positions was found (table 1) and since the causes of variation appeared to be of somewhat different magnitudes for the two positions, a covariance analysis was carried out to determine the genetic and environmental correlations. The form of the covariance analysis follows that of the variance analysis (table 3). The relationships between the causes of

TABLE 4

Values of components from variance and covariance analyses. The S component for line crosses contains differences between reciprocal line crosses as well as differences between unrelated crosses.

Component	Production-bred flock			Single sire inbred lines			Line crosses		
	Variance		Covariance	Variance		Covariance	Variance		Covariance
	Position 1	Position 2		Position 1	Position 2		Position 1	Position 2	
S	0.713	1.060	0.885	2.073	0.961	1.399	1.461	2.952	2.241
D	0.141	−0.492	−0.252	.625	0.710	.829	.074	−.065	−.401
P	6.073	6.485	6.262	4.235	4.195	4.115	3.585	3.405	3.465
U	4.058	3.518	0.612	4.254	5.504	.471	3.658	4.649	.083
$S+D+P$	6.927	7.053	6.895	6.933	5.866	6.343	5.120	6.292	5.305
$S+D+P+U$	10.985	10.571	7.507	11.187	11.370	6.814	8.778	10.941	5.388

TABLE 5

Estimates of heritabilities and environmental effects for the production-bred flock

Effect	Formula	Position 1	Position 2	Both Positions
$\hat{e}^2$	$\frac{u}{S+D+P+U}$	.369	.333	—
e^2	$\frac{P-(S+D)}{S+D+P}$	.753	.839	—
h^2	$\frac{2(S+D)}{S+D+P}$	.247	.161	.189
h_z^2	$\frac{Z(S+D)}{S+D+P+u/n}$	.191*	.129*	.167**
h_z^2	$\frac{Z(S+D)}{S+D+P+U}$	.156	.108	.124

*Two eggs
**Four eggs, two from each position.

variation and their effects within the individual are shown in figure 3. The paths for each position are the same as those shown in figure 2, the more complicated arrangement being due to the necessity of representing both positions of a clutch on the same diagram.

The values of the correlations r^G, r^E, and $r^{\hat{E}}$ were found to be .909, 1.013, and .162 respectively (table 6). The phenotypic correlation was .697.

INBRED LINES

Effects of selection. There appears to be no relationship between mean egg shape and the direction of selection for egg weight or egg number in the inbred lines (table 7). These lines became differentiated with respect to egg weight as a result of direct selection for egg weight in the *HW* and *LW* lines and as a result of correlated response of egg weight to selection for egg number in the *HN* and *LN* lines, the descending order being *HW*, *LN*, *HN*, and *LW* (Shultz, 1953). The order with respect to

egg shape is *HW*, *LW*, *LN*, and *HN* with the individual lines being mingled. Other investigators have also failed to find evidence of a correlation between egg weight and egg shape (Pearl and Surface, 1914; Benjamin, 1920; Marble, 1943).

Analysis of variance and covariance. Since no interaction between positions and other sources of variation was found in the production-bred flock, the analysis of variance of positions separately and the covariance analysis only were undertaken in the inbred lines. It should be noted that these lines are single sire lines and hence the between sire component also includes the difference between lines. The sire component in the case of crosses represents a pooling of the differences between reciprocal line crosses with differences between unrelated crosses. The values of the various components are given in table 4.

Interpretation of components. The interpretation of the components of variance obtained from inbred lines is different from that of larger random bred populations, such as the production-bred flock, due to the effect of random fluctuation of gene frequencies on the genetic variance (Dickerson, 1942). If there is no selection, then the proportion of the genetic variance contained in each of the components depends on the

TABLE 6

Correlations between first and second clutch positions. Components of covariances designated by subscript 12. Components of variance designated by subscript 1 or 2 for first and second positions respectively.

Correlation	Formula	Prod-Bred Flock	In-breds	Crosses
r^G	$\frac{S_{12}+D_{12}}{\sqrt{(S_1+D_1)(S_2+D_2)}}$	0.909	1.049	0.874
r^E	$\frac{P_{12}-S_{12}-D_{12}}{\sqrt{(P_1-S_1-D_1)(P_2-S_2-D_2)}}$	1.013	0.952	1.578
$r^{\hat{E}}$	$\frac{U_{12}}{\sqrt{U_1U_2}}$	0.162	0.097	0.020
r^k	$\frac{S_{12}+D_{12}+P_{12}}{\sqrt{(S_1+D_1+P_1)(S_2+D_2+P_2)}}$	0.997	0.995	0.935
r^Z	$\frac{S_{12}+D_{12}+P_{12}+U_{12}}{\sqrt{(S_1+D_1+P_1+U_1)(S_2+D_2+P_2+U_2)}}$	0.697	0.604	0.550

TABLE 7

Mean shape indexes $\left(\frac{100 \text{ breadth}}{\text{length}}\right)$ of inbred lines and line crosses. In crosses, sire's line is given first.

Line or Cross	Number of pullets	Position 1	Position 2
HNP	7	72.45	71.50
HNM	10	70.84	73.02
LNP	33	74.80	74.91
LNM	26	72.20	73.36
HWP	15	75.51	75.23
HWM	14	74.92	74.83
LWP	26	75.11	75.40
LWM	3	72.83	75.95
Weighted mean		73.99	74.40
Unweighted mean	134	73.58	74.28
HNP × *HNM*	25	72.33	71.84
HNM × *HNP*	14	72.30	72.30
LNP × *LNM*	17	73.93	74.32
LNM × *LNP*	9	71.28	71.94
HWP × *HWM*	22	75.45	76.83
HWM × *HWP*	20	74.72	74.84
LWP × *LWM*	9	75.02	75.76
LWM × *LWP*	38	74.14	74.88
Weighted mean		73.80	74.25
Unweighted mean	154	73.65	74.09
Prod-bred flock	498	73.55	73.87

TABLE 8

Values for the between pullet component of variance, P, for November egg weight and shank length in the 1950 generation inbreds and crosses.

Number of lines	Lines selected for	November egg weight		Shank length	
		Inbreds	Crosses	Inbreds	Crosses
2	High November egg weight	14.211	13.905	.1000	.0861
4	High or low November egg number	10.784	7.860	.0844	.0463
2	Low November egg weight	8.347	4.610	.0589	.0351

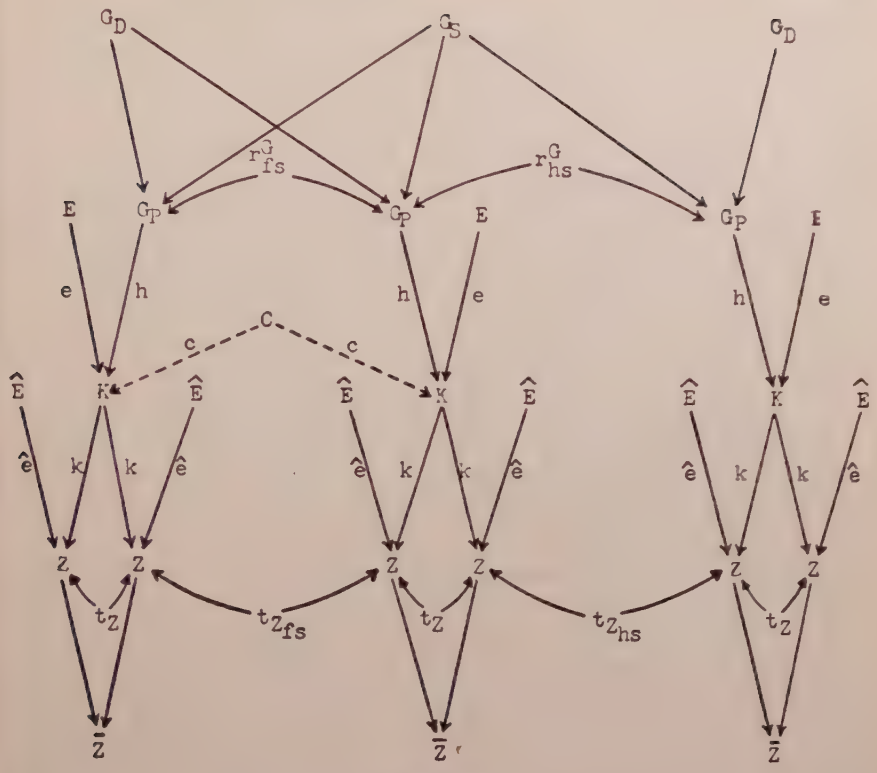

FIGURE 2.

PATH DIAGRAM FOR ONE CLUTCH POSITION. THERE WAS NO EVIDENCE OF MATERNAL EFFECTS (C). EXPLANATION OF SYMBOLS IN TEXT.

coefficients of inbreeding of the pullets (F) and of their parents (F'). Following Wright's (1921) methods of path coefficients, the genetic correlations due to autosomal and sex-linked genes were found to be as given in table 3.

In this table, F_{SL} and F'_{SL}, applying to sex-linked genes, are defined as the correlation between gametes each of which contain a sex chromosome. Hence, F'_{SL} is the inbreeding coefficient of the homogametic parents. It should be noted that the expected rate of increase in F will be different for autosomal and sex-linked genes (Crow and Roberts, 1950) so that F_A is not equal to F_{SL} and F'_A is not equal to F'_{SL}. If selection, natural or artificial, is acting to retard the increase in homozygosity due to inbreeding, the coefficients, F and F', calculated on the basis of relationship will be overestimated.

Under the assumption of additive gene action, the subdivision of a

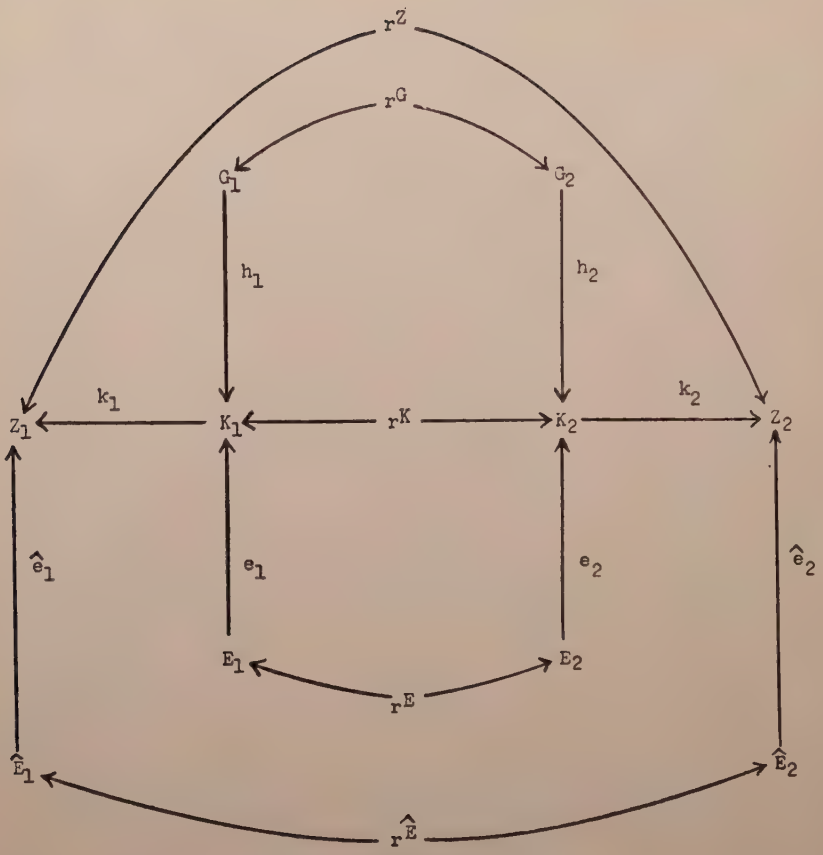

FIGURE 3.

PATH DIAGRAM SHOWING RELATIONSHIPS BETWEEN CLUTCH POSITIONS (1 AND 2) WITHIN CLUTCHES WITHIN PULLETS. EXPLANATION OF SYMBOLS IN TEXT.

population into a number of isolated lines (such as was done in effect in this material) will theoretically result in an increase in the total genetic variance by the factor $1 + F_A$ for autosomal genes and $1 + F_{SL}$ for sex-linked genes (homogametic sex). No increase will occur with respect to sex-linked genes in the heterogametic sex although the distribution of the genetic variance within and between lines will change. Considering only the homogametic sex, the genetic variance between lines increases as inbreeding occurs as illustrated by the expression

$$\frac{1 + F'_A + 6F_A}{4} \sigma^2_{G_A} + \frac{1 + F'_{SL}}{2} \sigma^2_{G_{SL}}$$

for component S in single sire lines. The genetic variance within single sire lines decreases in accordance with the expression

$$\frac{3 - F'_A - 2F_A}{4} \sigma^2_{G_A} + \frac{1 - F'_{SL}}{2} \sigma^2_{G_{SL}} .$$

Changes in the genetic variance due to random fluctuations of gene frequencies resulting in the differentiation of lines and an increase in genetic variance between lines has been called genetic drift, the possible evolutionary significance of which has been particularly elaborated by Wright (1931). These formulas (table 3) furnish a measure of the genetic drift expected in the absence of selection. However, if gene action is not additive, i.e. with dominance, the genetic variance would not behave in the above manner (Robertson, 1952).

Nothing is known about the effect of inbreeding on the magnitudes of the other causes of variance. In this material, maternal effects apparently are not present as shown by the analysis of the production-bred flock and hence can be ignored here. The magnitude of σ^2_E can be obtained directly and comparisons made between the production-bred flock, the inbred lines, and line crosses. An estimate of σ^2_E free from any effects of selection on the genetic variance cannot be obtained directly. However, the amount of genetic variance contained in the component P is the same for both inbreds and crosses, being dependent in both cases on the inbreeding of the parents only (table 3). Since the same sires were used in producing both inbreds and crosses, comparisons of the respective P components should give a good estimate of the relative effects of the environment E on inbreds and crosses.

Results. The inbred lines and the crosses between them show evidence of sex linkage. Thus the sire component is much larger than the dam component (table 4) the difference between them being greater than would be expected from the action of inbreeding on the genetic variance. This is in agreement with the findings in the production-bred flock discussed previously. The means of the crosses also indicate sex linkage, the order of reciprocal crosses between two inbred lines following the order of the sires' lines in all cases except for the second position in the crosses between *LWP* and *LWM*.

The differences between sires from different inbred lines is greater than the difference between sires in the relatively non-inbred production-bred flock (table 4). This divergence of the inbred lines must have been due to genetic drift since there appears to be no relationship between artificial selection as practiced in these lines and egg shape.

The difference between dams, contrary to expectations, is larger in the inbred lines than in the production-bred flock and line crosses

although still of rather small magnitude. This appears to be a reflection of sampling errors and the small amount of autosomal genetic variance.

The difference between pullets is less within inbred lines and their crosses than in the production-bred flock as expected on the assumption of genetic drift. However, P from the inbreds is greater than P from the crosses. Since the expected portion of the genetic variance contained in this component is the same in each case, the difference appears to be due to a greater effect of the environment (E in figures 2 and 3) on the inbreds than on the crosses.

The component U is largest in the inbreds, somewhat smaller in the production-bred flock, and smallest in the crosses. Since this is a within pullet component, and hence due entirely to environmental factors ($\hat{E}$ in figure 2 and 3), it appears that the inbreds are more sensitive and the crosses less sensitive to this source of environmental variation than the relatively non-inbred production-bred pullets.

CONCLUSIONS

The analysis of the production-bred flock brought to light three factors which need to be considered in a breeding program for egg shape. First, the heritability as determined for this flock is moderately low ranging from .11 for a single second position egg to .19 for a mean measurement of two first position eggs (table 5). These are intermediate values such that combined selection would be considerably more effective than straight family selection or individual selection alone. Second, the heritabilities and correlations suggest that the most gains in the index of either position would be obtained by selection on the basis of first position eggs alone. However, selection without regard to position is almost as effective and is much more feasible in practical breeding programs. Third, the presence of sex linkage in this flock was indicated by the analysis of the production-bred flock. The means and the analysis of variance of the crosses between inbred lines derived from the production-bred flock substantiate this conclusion. If all genes contributing to the variability of a character were sex-linked, full and half sister records would be of equal value, since females receive a sex chromosome from the sire but none from the dam. A progeny test of the females would add nothing since the correlation between dam and daughter due to sex-linked genes is zero. Information about the genetic worth of a cockerel with respect to the sex chromosome received from the sire can be obtained from his full and half sisters. A progeny test is necessary, to evaluate the sex chromosome received from his dam. Thus, under

conditions comparable to those found in this flock for egg shape, a full scale breeding program designed to alter the mean of a character inherited in this manner would call for combined individual and full and half sister selection of pullets with sister and progeny testing of sires. In the case of egg shape where the desirable value is an intermediate one, somatic disassortative mating (the mating of phenotypically unlike individuals) may be useful for production of commercial stock (Lerner, 1950).

Genetic drift (Wright, 1921, 1931) appears to have occurred in the inbred lines. Thus the difference between sires (S) from different lines is greater than the difference between sires in the relatively non-inbred production-bred flock (table 4). The component S is entirely genetic in composition (table 3) and is expected to increase as the coefficient of inbreeding increases. On the other hand, the difference between dams (D) appears to have increased with inbreeding contrary to expectations. Thus, both the production-bred flock and the crosses yield small positive values for the first position and negative values for the second position, whereas the inbred lines show sizable positive values. Whether this is the result of sampling errors due to the small number of dams involved or whether the genetic variance within inbred lines has actually increased is difficult to decide. Robertson (1952) has shown that if there is dominance, an increase in the genetic variance might be expected.

The inbreds seem to be more sensitive to environmental conditions than are the crosses or the random-bred individuals from the production-bred flock. Thus the difference between clutches within pullets, strictly an environmental component, is greatest for the inbreds, next largest for the production-bred flock, and smallest for the crosses. The between pullet component (P) is also useful in this connection if it is assumed that the genetic variance has decreased within lines according to the expectations given in table 3. The expected genetic composition of the P component is the same for inbreds and crosses. Hence, the greater value of P for the inbreds than for the crosses must reflect a greater environmental variance (σ_E^2) in the inbreds. The fact that the difference between pullets (whether inbreds or crosses) from inbred dams is less than the difference between random-bred pullets from the production-bred flock would be attributed to a decrease in the genetic variance within lines upon inbreeding. This phenomenon was noted in the analysis of two other characters (November egg weight and shank length) in these same inbreds and crosses, namely, that the difference between pullets was greater in the inbreds than in the crosses (table 8).

SUMMARY

1. An analysis of variance and covariance was carried out for egg shape $\left(\frac{100 \text{ width}}{\text{length}}\right)$ using the first and second position eggs of two clutches from each of 498 pullets in the U. C. production-bred flock, 134 pullets from eight inbred lines, and 154 pullets from crosses between these inbred lines.

2. No interactions between clutch positions and other sources of variance (sires, dams, pullets) were found.

3. The shape index for both clutch positions was greater in the second clutch than in the first clutch measured indicating that the shape index was increasing during the period in which measurements were taken (March and April). The difference was significant.

4. The shape index of the second position was significantly higher than the index of the first position. If the distinction between positions is disregarded, the consequent reduction in heritability is negligible.

5. Heritability estimates ranged from .11 on the basis of a single second position egg to .19 on the basis of the mean of two first position eggs. Heritability for the index of the first position was higher than that of the second position.

6. The estimate of the theoretical maximum heritability (an infinite number of eggs measured per individual) was .25 and .16 for the first and second positions respectively.

7. Evidence of sex linkage was obtained from the analysis of the production-bred flock and the crosses between inbred lines.

8. Under the above conditions, improvement of a character (expressed only in females) would be made most efficiently by the use of combined individual and sister (for sex-linked genes, full and half sister records are of equal value) selection of pullets and by the use of sister and progeny testing in the selection of males. In the case of sex linkage, daughters' records are of no value in the selection of dams.

9. The estimates of the components of correlation between first and second position indexes of the same bird and clutch in the production-bred flock are as follows: $r^{\hat{E}} = 0.162$, which is the correlation that would result were variation due only to environmental influence contributing to differences of individual eggs and different clutches of individual birds; $r^{E} = 1.013$, which is the correlation that would result were variation due only to those additional environmental influences contributing to variation between birds; and $r^{G} = 0.909$, which is the correlation that would result were variation due only to genetic differences between birds.

10. The eight inbred lines have become differentiated with respect to egg shape by genetic drift. No correlated response of egg shape to selec-

tion for November egg weight or egg number as practiced in these lines occurred.

11. Inbreds were found to be more sensitive and the crosses less sensitive to environmental effects than the more or less randomly bred birds of the production-bred flock. It was also noted that the inbreds appeared to be more sensitive to environmental variations than the crosses for November egg weight and shank length.

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A GENERALIZATION OF NEYMAN'S CONTAGIOUS DISTRIBUTIONS

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1. *Review of experience with Neyman's contagious distributions.*

Neyman (1939) presented a new class of contagious distributions applicable to situations where individuals or items are supposed initially dispersed in randomly scattered groups—egg masses in the case of insects or clumps in the case of bacteria—which are subject to a chance fluctuation in size. It may be supposed that there occurs subsequently some spacial dispersion from the initial groups. Under such a scheme, the distribution of individuals, or items, cannot be random. Since the probability of an individual occurring in a given unit area is related to the probability of other individuals occurring in that unit area, the distributions may be called contagious.

Upon the previous general grounds, Neyman has suggested three types of theoretical frequency distribution called Types A, B, and C. These types all correspond well to observed data for cases where the mechanism of dispersion is roughly that previously set forth. They proved better than a Poisson distribution and superior to the negative binomials, often used under such circumstances. The novel character of the new distributions may be seen, in the extreme, from the fact that they can be multimodal. This character is, of course, in violent contrast to that of the Poisson or negative binomial but yet corresponds very well with the biological reality. These conclusions were reached on various biological applications, as in the original work by Neyman (1939) and in work by one of the writers (Beall 1940) and by Archibald (1948). Often in this experience, Types A, B and C fit data progressively better although sometimes the sequence of types seems not to go far enough. For illustration, there is the case of *Pyrausta nubilalis* Hubn. in 1937 (Table VII, Beall 1940), where the types give values of χ^2 with progressively the probabilities of .011, .064 and .110. As a second illustration there is the case of *P. nubilalis* in the same source but which has been reproduced in the present discussion as Table II. It can be seen the values of χ^2 again fall and have progressively the probabilities of .05, .48 and .57.

2. *A general class of contagious distributions including those proposed by Neyman.*

The impression that Neyman's types do not go far enough is of interest in view of the fact that their form suggests they are simply early members of a great family of distributions, as will be shown immediately below. The nature of this family may be seen by starting with Types A, B and C as special cases.

Consider the characteristic functions of Neyman's types i.e.,

$$\left.\begin{aligned}
\varphi_A(t) &= \exp\{m_1\{e^{m_2(e^{it}-1)} - 1\}\} \\
\varphi_B(t) &= \exp\{m_1\{e^{m_2(e^{it}-1)} - 1 - m_2(e^{it}-1)\}/m_2(e^{it}-1)\} \\
\varphi_C(t) &= \exp\{2m_1\{e^{m_2(e^{it}-1)} - 1 - m_2(e^{it}-1) \\
&\qquad - m_2^2(e^{it}-1)^2\}/m_2^2(e^{it}-1)^2\}
\end{aligned}\right\} \tag{1}$$

where m_1 and m_2 are parameters, of which the numerical value is peculiar to the type. Equations (1) suggest* the general form

$$\left.\begin{aligned}
\varphi(t) &= \exp\left\{m_1\Gamma(n+1)\sum_{s=0}^{\infty}\frac{m_2^{s+1}(e^{it}-1)^{s+1}}{\Gamma(n+s+2)}\right\} \\
&= \exp\{-m_1\}\exp\left\{m_1\Gamma(n+1)\sum_{s=0}^{\infty}\frac{m_2^s(e^{it}-1)^s}{\Gamma(n+s+1)}\right\}
\end{aligned}\right\} \tag{2}$$

where the special cases of (1) arise for $n = 0$, 1 and 2. For $n > 2$ we should have new distributions. For $n < 2$ and not an integer, we should have cases intermediate to Neyman's A, B and C.

We note that $\varphi(t)$ of (2) looks like a characteristic function and applying the general operation of finding quantities a_r from expansion into the Maclaurin series of

$$\varphi(t) = \sum_{r=0}^{\infty} a_r e^{itr} \tag{3}$$

we note first that

$$\sum_{r=0}^{\infty} a_r = \varphi(0) = 1 \tag{4}$$

which is a necessary condition for $\varphi(t)$ to be a characteristic function. This means, of course, that if $\varphi(t)$ is a characteristic function and the quantities a_r are the probabilities of 0, 1, 2, etc., observations, then as is necessary they total to unity. It is however, further necessary to show

*An alternate way of arriving at the generalization (2) is suggested in an Appendix to the present communication.

that a_r is nonnegative for all r, which is a more lengthy matter. This second condition means, of course, that in a true probability distribution, all the quantities a_r , being probabilities, are nonnegative.

To prove the second point let

$$f(e^{it}) = \Gamma(n+1) \sum_{s=0}^{\infty} \frac{m_2^s (e^{it}-1)^s}{\Gamma(n+s+1)} \tag{5}$$

whence and from (2), we have the generalized function

$$\varphi(t) = e^{-m_1} e^{m_1 f(e^{it})} \tag{6}$$

Let us note that

$$\frac{d\varphi}{de^{it}} = m_1 \varphi f' \tag{7}$$

so that

$$\begin{aligned} a_{r+1} &= \frac{d^{r+1}\varphi}{d(e^{it})^{r+1}}\bigg|_{e^{it}=0} = \frac{m_1}{(r+1)!} \frac{d^r}{d(e^{it})^r} \{f'\varphi\}\bigg|_{e^{it}=0} \\ &= \frac{m_1}{r+1} \sum_{k=0}^{r} \frac{1}{k!} \left\{\frac{d^{k+1}f}{d(e^{it})^{k+1}}\right\} \frac{1}{(r-k)!} \frac{d^{r-k}\varphi}{d(e^{it})^{r-k}}\bigg|_{e^{it}=0} \\ &= \frac{m_1}{r+1} \sum_{k=0}^{r} \frac{1}{k!} f^{(k+1)}(0) a_{r-k} \end{aligned} \tag{8}$$

where it can be seen that any value a_r is expressible in terms of a_0 through a_{r-1} , by means of the recurrent coefficients $f^{(k+1)}(0)\ /k!$ and so if these are always nonnegative and a_0 is nonnegative all a_r will be nonnegative.

In order to show $f^{(k+1)}(0)$ is always positive it is easy to see from a simple division, by the power series of e^{-m_2}, that

$$f^{(k+1)}(0) = e^{-m_2} \sum_{s=0}^{\infty} \frac{n\Gamma(n+s)(k+1)!\, m_2^{s+k+1}}{s!\Gamma(n+s+k+2)} \tag{9}$$

where the first factor is intrinsically nonnegative and the second is nonnegative whenever $m_2 > 0$, since n, k and s are taken always nonnegative. It is at once obvious that $a_0 = \varphi(0)$ is necessarily nonnegative and therefore, in connection with (9), every a_r is nonnegative. Returning to (3) it is now plain that $\varphi(t)$ has the necessary and sufficient characters to be regarded as a characteristic function. We have, accordingly, obtained a generalization of Neyman's contagious distributions characterized by a critical parameter which we shall call $0 \leq n \leq \infty$ and not necessarily an integer. For his original Type A, $n = 0$, for B, $n = 1$ and for C, $n = 2$.

3. *Calculation of the frequency distribution*

Following general procedure with characteristic functions, for any distribution, the probability of an observation of r events is

$$P_r = a_r \tag{10}$$

which involve operations with e^{it}, and this, for convenience, we shall replace by z. We turn from the characteristic function $\varphi(t)$ of (2) to the generating function

$$\psi(z) = \exp\{-m_1\} \exp\left\{m_1\Gamma(n+1) \sum_{s=0}^{\infty} \frac{m_2^s(z-1)^s}{\Gamma(s+n+1)}\right\} \tag{11}$$

or for n an integer

$$\psi(z) = \exp\{-m_1\} \exp\left\{m_1 n! \sum_{s=0}^{\infty} \frac{m_2^s(z-1)^s}{(s+n)!}\right\} \tag{12}$$

Then

$$P_r = \frac{1}{r!} \frac{d^r}{dz^r} \psi(z)\Big|_{z=0} \tag{13}$$

Setting in (12), $n = 0$, 1 or 2, successively we obtain the generating functions of Neyman (1939) for Types A, B and C, respectively.

In calculating one finds from (5) and (13)

$$P_0 = e^{-m_1} e^{m_1 f(0)} \tag{14}$$

Then from (10) and (8), conveniently

$$P_{r+1} = \frac{m_1}{(r+1)} \sum_{k=0}^{r} F_k P_{r-k} \tag{15}$$

where, we let

$$F_k = \frac{1}{k!} f^{(k+1)}(0) \tag{16}$$

This recurrent relationship is that originally derived by Beall, as discussed by Neyman (1939) in work on the latter's Type A. Even the recurrent operation of (16) that involves us in repeated differentiation of

$$f(z) = n! \sum_{s=0}^{\infty} \frac{m_2^s(z-1)^s}{(n+s)!} \tag{17}$$

i.e.,

$$f^{(k)}(0) = n! m_2^k \sum_{s=0}^{\infty} \frac{(s+k)!(-m_2)^s}{s!(n+s+k)!} \tag{18}$$

can, itself, however, prove difficult and tedious. Equation (18) is generally hard to evaluate so that it is worth while to point out three methods for its determination in any practical application.

One method, that suggests itself, for finding successive values of $f^{(k+1)}(0)$ is to note that

$$f^{(k+1)}(0) = m_2 f^{(k)}(0) + n \sum_{t=0}^{k} \frac{k!}{(k-t)!} f^{(k-t)}(0) - k!n \tag{19}$$

as can be proven readily enough by replacing the expressions f by the general form of (18) and collecting on powers of $(-m_2)$. The successive values F_k are readily obtained from (16).

A second method for successive values of $f^{(k+1)}(0)$, which is the best in common practice, notes first that

$$\begin{aligned} f(0) &= n! \sum_{s=0}^{\infty} \frac{(-m_2)^s}{(n+s)!} \\ &= n!(-m_2)^{-n}\left\{e^{-m_2} - \sum_{s=0}^{n-1} \frac{(-m_2)^s}{s!}\right\} \end{aligned} \tag{20}$$

secondly that

$$\begin{aligned} f'(0) &= n!m_2 \sum_{s=1}^{\infty} \frac{s(-m_2)^{s-1}}{(n+s)!} \\ &= n!m_2 \sum_{s=1}^{\infty} \frac{(-m_2)^{s-1}}{(n+s-1)!} - n!nm_2 \sum_{s=0}^{\infty} \frac{(-m_2)^{s-1}}{(n+s)!} - n \\ &= (m_2+n)f(0) - n \end{aligned} \tag{21}$$

and finally that for $k > 1$, from (18) by rearrangement,

$$f^{(k+1)}(0) = (m_2 + n + k)f^{(k)}(0) - km_2 f^{(k-1)}(0) \tag{22}$$

From the foregoing and (16), values of F_k are obtained, for example,

$$F_0 = f'(0) \tag{23}$$

and for $k > 1$

$$F_k = \frac{m_2 + n + k}{k} F_{k-1} - \frac{m_2}{k} F_{k-2} \tag{24}$$

The procedure just recommended, and in particular the use of (24), is a very rapid and convenient way of getting the necessary values F in (15) but does suffer from the defect in actual calculation that with successive steps rounding error in the earlier values, F, creeps towards the left of later values of F. There seems to be a tendency for the successive values, F, to lose almost a digit, so far as

accuracy is concerned, at each step so that even if $f(0)$ is found with ten figure accuracy one can only go so high as to F_9 or F_{13}. This tendency becomes more acute as n gets great. These shortcomings demand, for situations where the values of F_k do not converge fairly rapidly, the development of yet a third method of finding the values of F, as is set forth immediately below. This third method is a little longer than that commonly recommended but is reliable to very high values of k.

The recurrent coefficients may be conveniently calculated, for cases where n is an integer, by a method other than that previously employed. Consider the convenient quantities

$$ {}_n\theta_k = \frac{f_n^{(k)}(0)}{k!} = \frac{n!m_2^k}{k!} \sum_{s=0}^{\infty} \frac{(s+k)!(-m_2)^s}{(n+s+k)!s!} \tag{25} $$

where $f_n^{(k)}(0)$ is the value of $f^{(k)}(0)$, in the sense of (19), for any given value of n. Let us consider the first negative forward difference,

$$ \begin{aligned} -\Delta_{1,k} &= {}_n\theta_k - {}_n\theta_{k+1} \\ &= \frac{n!\, m_2^k}{(k+1)!} \sum_{s=0}^{\infty} \frac{\{s+(k+1)\}!(-m_2)^s}{\{s+(k+1)-(n-1)\}!s!} \\ &= \frac{n}{m_2}\, {}_{(n-1)}\theta_{k+1} \end{aligned} \tag{26} $$

Plainly the second negative forward difference is

$$ \begin{aligned} -\Delta_{2,k} &= -\Delta_{1,k} + \Delta_{2,k} \\ &= \frac{n}{m_2} \{ {}_{(n-1)}\theta_{k+1} - {}_{(n-1)}\theta_{k+2} \} \\ &= n(n-1)m_2^{-2}\, {}_{(n-2)}\theta_{k+2} \end{aligned} \tag{27} $$

and the r-th negative forward difference is, by extension of such operation,

$$ -\Delta_{r,k} = \frac{n!m_2^{-r}}{(n-r)!}\, {}_{(n-r)}\theta_{k+r} \tag{28} $$

Accordingly, whatever the value of n (an integer) it is possible to obtain a sufficiently high negative forward difference so that there is involved

$$ \begin{aligned} -\Delta_{n,k} &= n!m_2^{-n}\, {}_0\theta_{k+n} \\ &= \frac{n!}{m_2^n} \frac{f_0^{(k+n)}}{(k+n)!} \\ &= \frac{n!m_2^k}{(k+n)!} e^{-m_2} \end{aligned} \tag{29} $$

These differences are easily calculated and are fairly rapidly convergent, so that the series of ${}_n\theta_k$ can be conveniently built back.

Let us not consider the obvious way of employing the previous results, i.e., by writing down a column of easily calculated negative forward differences, $-\Delta_{n,k}$, neglecting all differences after they become vanishingly small, and building back. This procedure becomes heavy as m_2 gets great and the table must be made very long, in order to get reasonable accuracy.

An alternate, less obvious but more practical way of employing the previous results involves accumulation down the columns so that the table may be made as short or as long as desired. It is necessary to invoke for $k < 0$, values

$$\begin{aligned} {}_n\theta_k &= \frac{n!m_2^k}{k!} \sum_{s=0}^{\infty} \frac{(s+k)!(-m_2)^s}{(s+k+n)!s!} \\ &= \frac{n!m_2^k}{k!} \sum_{s=0}^{-k+1} \frac{(s+k)!(-m_2)^s}{(s+k+n)!s!} \end{aligned} \tag{30}$$

so that, conveniently,

$$\left.\begin{aligned} {}_n\theta_{-1} &= nm_2^{-1} \\ {}_n\theta_{-2} &= n(n-1)m_2^{-2} + nm_2^{-1} \\ {}_n\theta_{-3} &= n(n-1)(n-2)m_2^{-3} + 2n(n-1)m_2^{-2} + nm_2^{-1} \end{aligned}\right\} \tag{31}$$

etc. From the definition (30), the results of (26) to (29), of course apply. Accordingly, it is possible to build up a series of values ${}_n\theta_k$ for all k very conveniently, thus for instance, suppose we want to calculate ${}_2\theta_k$. We may construct Table I which is built downwards from these results. The values of $f_n^{(k)}(0)$ or of F_k may again be found very simply from those for ${}_n\theta_k$.

4. *The character of various contagious distributions, n an integer*

Let us consider the character of the generalized contagious distribution. First we shall write from the characteristic function, (2) and from the rule that the r-th moment about origin of a distribution with characteristic function $\phi(t)$ is obtained from

$$i^r\mu_r' = \left.\frac{d^r\phi(t)}{dt^r}\right|_{t=0} \tag{32}$$

that

TABLE I. AN ILLUSTRATION FOR $n = 2$ OF THE DIFFERENCE METHOD FOR GETTING ${}_2\theta_k$.

k	${}_2\theta_k$	$-\Delta_{1,k}$	$-\Delta_{2,k}$
-2	$\frac{2}{m_2^2} + \frac{2}{m_2}$		
-1	$\frac{2}{m_2}$	$\frac{2}{m_2^2}$	
0			$2!m_2^{-2}e^{-m_2}$
1	These	values	$2!m_2^{-1}e^{-m_2}$
2	may	be	e^{-m_2}
3	built	up	$\frac{2!m_2}{3!}e^{-m_2}$
4	from the	numerical	$\frac{2!m_2^2}{4!}e^{-m_2}$
etc.	equivalents of	those shown	etc.

$$\left.\begin{aligned} \mu_1' &= m_1 m_2/(n+1) \\ \mu_2 &= \frac{m_1 m_2}{n+1}\left(1 + \frac{2m_2}{n+2}\right) \\ \mu_3 &= \frac{m_1 m_2}{n+1}\left\{1 + \frac{6m_2}{n+2} + \frac{6m_2^2}{(n+2)(n+3)}\right\} \end{aligned}\right\} \tag{33}$$

from (21) we see that

$$\mu_2 - \mu_1' = 2m_2\mu_1'/(n+2) \tag{34}$$

so that the second moment, μ_2, will always be greater than μ_1', under our conditions that n and m_2 are positive. We see also that $m_1 \geq 0$ from consideration of μ_1' in (33). Also, $\mu_3 > \mu_2$.

From (33),

$$\left.\begin{aligned} m_2 &= (n+2)(\mu_2 - \mu_1')/2\mu_1' \\ m_1 &= (n+1)\mu_1'/m_2 \end{aligned}\right\} \tag{35}$$

and again, since generally n is a parameter, giving the type of distribution,

$$n = \frac{6(\mu_2^2 + \mu_1'\mu_2 - \mu_1'\mu_3 - \mu_1'^2)}{\mu_1'^2 + 2\mu_1'\mu_3 - 3\mu_2^2} \tag{36}$$

The results, (35) and (36), will be later used in the practical estimation of m_1 , m_2 and n from empirical moments, along the lines of the procedure of Neyman (1939) and Beall (1940). These results have, however, other uses in the meantime.

In certain connections, it is of interest to note that P_0 always decreases as n increases from zero towards infinity, provided that m_1 and m_2 are chosen, as in the work of Neyman (1939), Beall (1940) and subsequent writers, so that μ_1' and μ_2 are constant, i.e., identical with the empirical values. To see this point, note first from (14) that

$$\log P_0 = -m_1\{1 - f_n(0)\} \tag{37}$$

$$= -m_1 e^{-m_2} \sum_{s=0}^{\infty} \frac{m_2^{s+1}}{(n+s+1)s!} \tag{38}$$

and from (35) that

$$\left.\begin{aligned} \frac{dm_1}{dn} &= m_1/(n+1)(n+2) \\ \frac{dm_2}{dn} &= m_2/(n+2) \end{aligned}\right\} \tag{39}$$

so that by simple but extensive operation

$$\frac{d \log P_0}{dn} = \frac{m_1 e^{-m_2}}{(n+1)(n+2)} \sum_{s=0}^{\infty} \frac{m_2^{s+1}}{(s-2)!(n+s)(n+s+1)^2} \tag{40}$$

so that P_0 continually decreases as n increases, while m_1 and m_2 are positive, under which general condition we work.

Let us consider the limiting form of the generalized distribution as

$$n \to \infty \tag{41}$$

We have from (35)

$$m_2 = \frac{n+2}{2} \frac{\mu_2 - \mu_1'}{\mu_1'} = (n+2)\frac{c}{2} \tag{42}$$

and substituting (42) in (20) we have

$$f_n(0) = n! \sum_{s=0}^{\infty} \frac{(n+2)^s}{(n+s)!} \left(-\frac{c}{2}\right)^s \tag{43}$$

In connection with the righthand member of (43), we may, as can be proven easily enough by simplification, note that, for the first k terms,

$$n! \sum_{s=0}^{k} \frac{(n+2)^s}{(n+s)!} \left(-\frac{c}{2}\right)^s = \frac{2}{2+c} \left\{ 1 + \sum_{s=1}^{k} \frac{n!(n+2)^{s-1}(2-s)}{(n+s)!} \left(-\frac{c}{2}\right)^s - \frac{n!(n+2)^k}{(n+k)!} \left(-\frac{c}{2}\right)^{k+1} \right\} \tag{44}$$

hence, under (41),

$$f_n(0) \to \frac{2}{2+c} \tag{45}$$

for all c.

Now from (35), under (41)

$$m_1 \to \frac{2}{c} \mu_1' \tag{46}$$

Accordingly

$$\begin{aligned} P_0 &= \exp \{ m_1 \{ f_n(0) - 1 \} \} \\ &\to \exp \left\{ -\frac{2\mu_1'}{2+c} \right\} \end{aligned} \tag{47}$$

Consider under (41), $n \to \infty$, now the general value of $f_n^{(k)}(0)$ which is done most conveniently from the consideration of the quantities ${}_n\theta_k$ of (25). Write from (26),

$${}_n\theta_k - {}_n\theta_{k+1} = \frac{n}{m_2} {}_{(n-1)}\theta_{k+1} \tag{48}$$

and note that

$${}_{(n-1)}\theta_{k+1} \to {}_n\theta_{k+1} \tag{49}$$

from (25) because the series involved is convergent for m_2 finite and

$$(n-1)!/(n+s+k-1)! \to n!/(n+s+k)!. \tag{50}$$

So from (48) and (49) we may write that under (41) and from (42),

$$\begin{aligned} {}_n\theta_k - {}_n\theta_{k+1} &\to \frac{n}{m_2} {}_n\theta_{k+1} \\ &\to \frac{2}{c} {}_n\theta_{k+1} \end{aligned} \tag{51}$$

so

$${}_n\theta_{k+1} \to \frac{c}{2+c} {}_n\theta_k \tag{52}$$

But since from (45) and (25)

$$ {}_n\theta_0 \rightarrow \frac{2}{2+c} \tag{53} $$

we can write that

$$ {}_n\theta_{k+1} \rightarrow \left(\frac{c}{2+c}\right)^{k+1} \frac{2}{2+c} \tag{54} $$

and from (25) in conjunction with (16) under (41)

$$ F_k \rightarrow (k+1)\left(\frac{c}{2+c}\right)^{k+1} \frac{2}{2+c} \tag{55} $$

We may note that (55) is convergent for $c \geq 0$; for the contagious distribution

$$ 0 \leq \frac{c}{2c} \leq 1 \tag{56} $$

Using (55), (47) and (46) in (15) it is very simple to get the frequency distribution as $n \rightarrow \infty$. The routine is discussed at some length in Section 7.

It may be of interest to note the moments of the contagious distribution under (41), as from (33), or in particular the relationship of the third moment to lower moments, i.e.,

$$ \begin{aligned} \mu_3 &= \mu_1'\left\{1 + 3c + \frac{3}{2}\frac{n+2}{n+3}c^2\right\} \\ &\rightarrow \mu_1'\left(1 + 3c + \frac{3}{2}c^2\right) \end{aligned} \tag{57} $$

We may compare (57) with the situation obtaining for a binomial, i.e.,

$$ \mu_3 = \mu_1'(1 + 3c + 2c^2) \tag{58} $$

where c has the meaning implicit in (42). The comparison of (57) with (58) may be of interest because it shows that even in the limit the new series of contagious distributions does not become identical with any binomial. The question might arise because obviously as from Fig. 1 and 2, the limit resembles at least superficially, say, a negative binomial much more than do the early cases like $n = 0$ and $n = 1$. The question is of importance because Wadley (1950)* has pointed out that in many cases where a contagious distribution might have been anticipated, a negative binomial gives a better representation than at least the case of $n = 0$. His work might be very profitably extended to

*While the present work was in press, Bliss, C. I. and R. A. Fisher, *Biometrics 9:* 176-200, have presented a further discussion in the same vein as that of Wadley.

a more general comparison of the negative binomial with contagious distributions.

5. *Applicability of the generalization in practice*

The foregoing generalization of Neyman's contagious distributions will now be considered from the point of view of how well it agrees with field observation. Perhaps we should say cases will be considered where there is some reason to suppose that a better correspondence will be found between data and theory when $n > 2$, i.e., the case lies beyond Type C in Neyman's terminology. A few other interesting cases will be considered. Only values of n an integer will be considered although intermediate, non-integer cases exist and are very similar. For all cases the primary data are presented. There are also shown fitted theoretical distributions for various low values of n and for $n \to \infty$. The n values have been assumed but the necessary particular values of m_1 and m_2 have been found by the method of moments, as in (35). The possibility of the very interesting refinement to a maximum likelihood solution as presented by Shenton (1949) for the case of $n = 0$ has not been considered because it is extremely laborious even in this the most simple situation. It would be impossibly laborious for our fairly extensive exploration for various values of n.

To begin, let us examine as in Table II and Fig. 1, the data of Beall

TABLE II. OBSERVED FREQUENCY OF *P. nubilalis* IN 1936 (BEALL 1940) AND CONTAGIOUS DISTRIBUTIONS WITH VARIOUS n.

In-sects	Obs.	Type A $n=0$	Type B $n=1$	Type C $n=2$	$n=3$	$n=4$	$n=5$	$n=6$	$n=7$	$n=8$	$n\to\infty$
0	24	27.0	24.5	23.4	22.8	22.4	22.2	22.0	21.9	21.7	20.8
1	6	1.5	4.0	4.9	5.4	5.7	5.9	6.0	6.1	6.2	6.9
2	4	3.0	4.1	4.6	4.9	5.0	5.1	5.2	5.3	5.3	5.7
3	5	4.1	4.1	4.2	4.3	4.4	4.4	4.4	4.5	4.5	4.7
4	3	4.3	3.8	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.8
5	2	3.8	3.2	3.2	3.1	3.1	3.1	3.1	3.1	3.1	3.1
6	1	3.0	2.8	2.7	2.6	2.6	2.5	2.5	2.5	2.5	2.4
7	1	2.3	2.2	2.2	2.1	2.1	2.0	2.0	2.0	2.0	1.9
8	2	1.7	1.8	1.7	1.7	1.6	1.6	1.6	1.6	1.6	1.5
9	1	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.2	1.2	1.2
10	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.9	.9	.9
11	3	.8	.8	.8	.8	.8	.8	.8	.7	.6	.7
12	1	.6	.6	.6	.6	.6	.6	.6	.5	.5	.6
13+	1	1.6	1.8	1.7	1.7	1.7	1.8	1.8	2.0	2.2	1.8
χ^2		12.80	5.49	4.81	4.60	4.62	4.62	4.69	5.71	6.23	6.21
P_{χ^2}		.05	.48	.57	.60	.60	.60	.58	.46	.40	.40

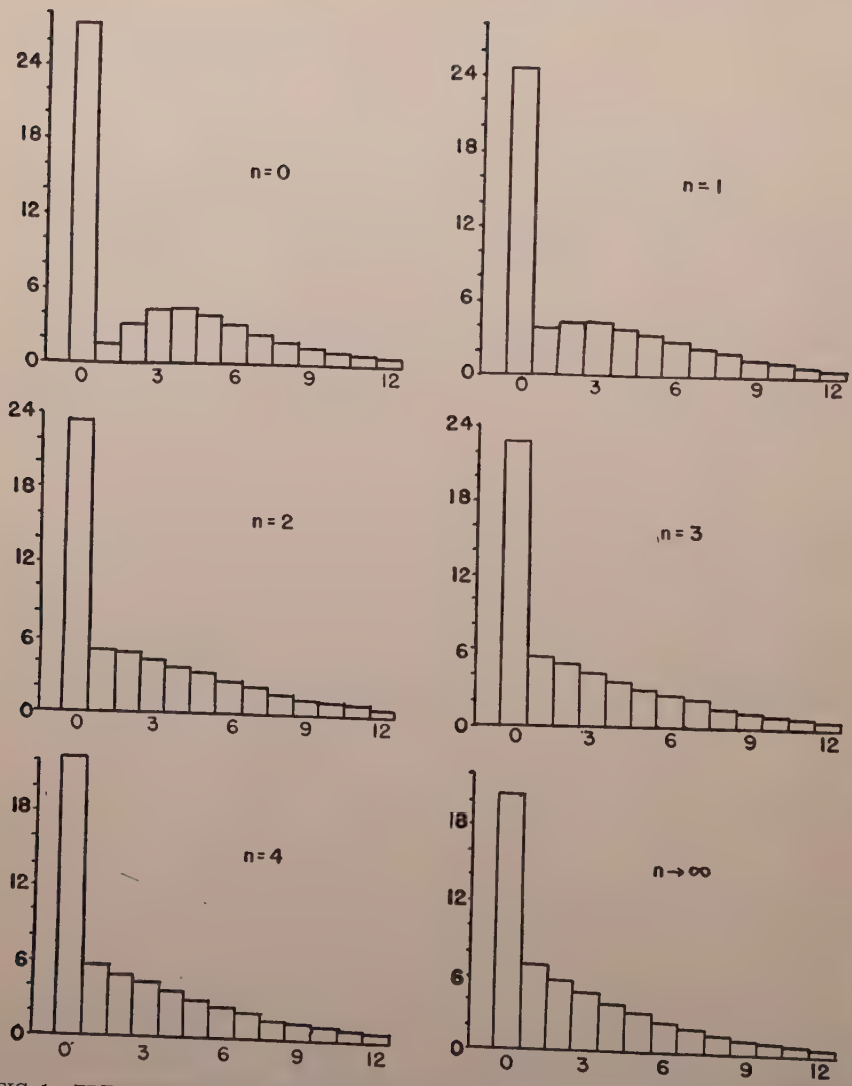

FIG. 1. FREQUENCY OF OBSERVATIONS 0, 1, 2, ETC. WITH VARIOUS n FOR *P. nubilalis*, AS IN TABLE II.

(1940) on the frequency of the European corn-borer, *Pyrausta nubilalis* Hubn., on small unit areas of a field as observed in 1936. It can be seen that for various values of n (the first and second moments constant) in the present case there is not exhibited the potential multimodality, apart from the bimodality that occurs for $n = 0$ and 1. The tail of the distributions is remarkably stable. Indeed, the main change that

occurs is a diminution of P_0 and a compensating augmentation of P_1 with some slight disturbance up to P_5 or P_6. The pace of such change is great for the early values of n but very small when n becomes substantial. The change in χ^2 reflects, of course, the situation just discussed, in that it changes rapidly at first and then increasingly slowly. Consideration of the value of χ^2, or better of its probability shown in the table, confirms our initial suspicion that the improvement apparent previous to the present investigation, as one goes from the case of $n = 0$ to the case of $n = 2$, continues, in some measure for higher n. In fact $n = 3$ to $n = 5$ gives the best fit. As n further increases the fit deteriorates slightly.

The senior author (Beall 1940) presented comparable data for several years on *P. nubilalis* for which the situation is very much the same in each year, so that they have not been considered at such length as the data of 1936. Only for 1937 is the situation considered briefly, as in Table III. The results, like those for 1936, but in more extreme form, are typical of the situation that prompted the present investigation, i.e., the correspondence of theory and observation seemed to improve markedly as n increased to 2; but 2 seemed not enough. The fits for $n = 8$ and $n \to \infty$ have been added and improve markedly to become quite good. Further cases were not studied because it appears that the case of $n \to \infty$ is probably the best. This conclusion is reached from the consideration of the contribution to χ^2 from various parts of the distribution. First, we can see that the theoretical P_0 drops always towards the observed proportion steadily and continuously {Equ. (40)} as n increases. Secondly, the theoretical P_1 rises steadily towards the observation. Thirdly, the contribution to χ^2 from the remaining classes P_2, P_3, etc., are very much the same for all n. It should be noted that here the results are to be contrasted with those for 1936 where n was best short of ∞.

One more example may perhaps be profitably considered from the work of Beall (1940), that of data on the potato beetle, *Leptinotarsa decemlineata*. Say, (Treatment 3 of the original paper) which had previously proven ill-fitted by $n = 0$, 1 and 2. In Table III $n \to \infty$ is added. The fit may be conceived improved but is still poor. As was discussed in the original paper, the potato beetles were at various stages of development and hence we may suppose that various contagious distributions were superimposed and our present simple theory is hardly applicable.

It should be noted that in some of the work of Beall (1940), $n = 0$ fitted well. We have picked cases mainly where $n = 0$ did not work. Thus in four series on *Loxostege sticticalis* L., $n = 0$ gave effectively a

TABLE III. OBSERVED FREQUENCY OF *P. nubilalis* IN 1937 OR *L. decemlineata* (BEALL 1940) AND CONTAGIOUS DISTRIBUTIONS WITH VARIOUS n.

Pyrausta nubilalis

Insects	Obs.	$n = 0^*$	$n = 1^*$	$n = 2^*$	$n = 8$	$n \to \infty$
0	33	37.8	37.1	36.8	36.1	35.7
1	12	5.6	6.8	7.3	8.3	8.8
2	6	5.2	5.0	5.0	5.0	5.1
3	3	3.5	3.2	3.1	2.9	2.9
4	1	1.9	1.9	1.8	1.7	1.6
5+	1	2.0	2.0	2.0	2.0	1.9
χ^2		9.04	5.57	4.47	2.90	2.17
$P\chi^2$		.011	.064	.11	.24	.34

Leptinotarsa decemlineata

Insects	Obs.	$n = 0^*$	$n = 1^*$	$n = 2^*$	$n \to \infty$
0	33	47.7	44.6	43.2	39.5
1	12	.4	2.7	3.7	6.0
2	5	1.1	2.8	3.4	4.9
3	6	2.0	2.8	3.2	3.9
4	5	2.8	2.8	2.8	3.2
5	0	3.2	2.6	2.5	2.5
6	2	3.0	2.4	2.2	2.0
7	2	2.5	2.1	1.9	1.6
8	2	1.9	1.7	1.6	1.3
9	0	1.4	1.4	1.3	1.0
10	1	1.0	1.0	1.0	.8
11+	2	3.0	3.1	3.2	3.3
χ^2		—	—	29.12	12.26
$P\chi^2$		—	—	.000,061	.06

*Fit as in Beall (1940).

better fit than $n = 1$ and this a better fit than $n = 2$. In each of these cases we note that P_0 was lower than the observed initial frequency. In accordance with (40) this tends to explain why higher n were not helpful.

Consideration of the change in character of the various contagious distributions in Table II, suggests that a case with some $n > 0$ may solve the problem that vexed Fracker & Brischle (1944). They worked with the frequency of plants, *Ribes* spp., in quadrats. They found that the case of $n = 0$ (Type A) was vastly better than the Poisson, but seemed to go too far and considered something intermediate between Type A and the Poisson. The higher members of the family as proposed in the present paper probably meet their problem. In illustration we have fitted for their case at Kaniksu, Idaho, for the $n \to \infty$ which does well as in Table IV.

Consideration of the P_0 and P_1 indicates that no finite n would do better. As a second illustration from their work, there is presented data from Clearwater, Idaho, using their 8% sample (they used the data on the 4% sample). Again the case of $n \to \infty$ gives a fair fit. In this case we have calculated for $n = 0$, which is inferior.

In Table V, there is considered the fit for $n \to \infty$ on the plant *Lespedeza capitata* as reported by Thomson (1952), it can be seen that there is some improvement over the situation for $n = 0$, although even for the latter the value of χ^2 would plainly be significant. We have added the maximum likelihood solution (following Shenton 1949) which seems to have repaid poorly all the trouble of its calculation. This is probably to be expected because from the nature of the solution it is directed towards the estimation of parameters and not at obtaining a theoretical distribution in agreement with observations.

One of the most successful applications of Neyman's contagious distributions was made by Archibald (1948) who studied the variation of various plants on small unit areas. This success doubtlessly occurred because the essential physical conditions underlying the variation in the plants were those originally considered by Neyman. There was involved an initial random distribution of mother plants from which there arose families of various size scattered about the site of the mother plant. For a number of species, Archibald fitted for $n = 0$, and this case only. For most species the fit was satisfactory but for one *Salicornia stricta* it was reported very poor (the χ^2 had a probability of .01) and we have considered this situation closely. It appears, however, in the first place, that some slip, on the part of Archibald, is involved, as is shown in Table VI, where $\chi^2 = 18.67$ and its probability is .18. We have considered $n = 0$ and 5 and $n \to \infty$. It appears that the fit becomes progressively better. It is probable that Archibald would also have gotten even better fits for at least some of the other species if she had used $n > 0$. This result is interesting because in this particular realm of botanical colonization, Thomas (1949) has suggested a dis-

TABLE IV. OBSERVED FREQUENCY OF *Ribes* (FRACKER & BRISCHLE 1944) AND CONTAGIOUS DISTRIBUTIONS

KANIKSU, IDAHO (4% SAMPLE)

Ribes	Obs.	$n = 0$*	$n \to \infty$
0	43	50.4	46.2
1	15	5.9	11.9
2	8	7.2	7.9
3	6	6.0	5.1
4	3	4.1	3.3
5	4	2.5	2.1
6	0	1.0	1.3
7+	1	3.1	2.2
χ^2		17.58	3.45
$P\chi^2$		.000,55	.33

CLEARWATER, IDAHO (8% SAMPLE)

Ribes	Obs.	$n = 0$	$n \to \infty$
0	26	41.1	31.7
1	9	.1	4.6
2	7	.3	4.0
3	3	.7	3.5
4	4	1.2	3.0
5	2	1.9	2.6
6	1	2.4	2.2
7	3	2.7	1.9
8	1	2.6	1.6
9	0	2.3	1.4
10	1	1.8	1.2
11	0	1.4	1.0
12	1	1.0	.8
13	1	.8	.7
14	2	.6	.6
15	1	.5	.5
16	1	.5	.4
17+	1	2.1	2.3
χ^2		68.23	7.24
$P\chi^2$		.000,000	.066

*Fit of Fracker and Brischle (1944).

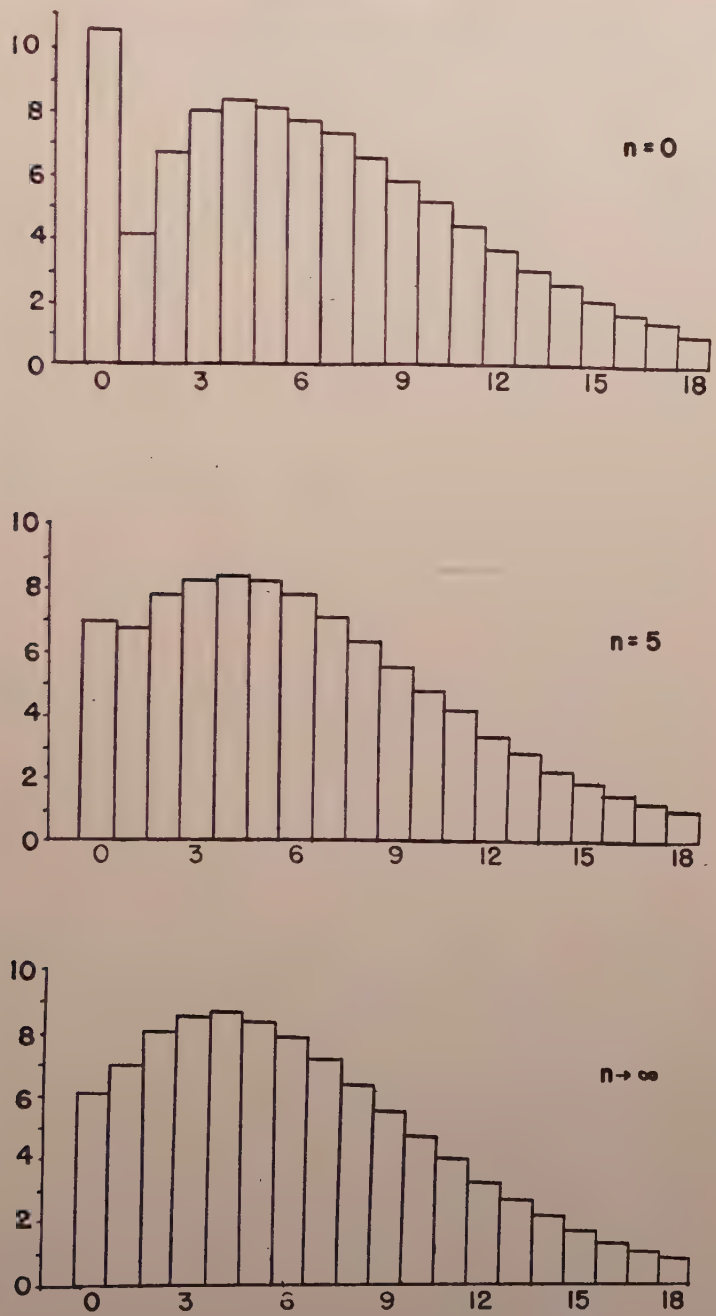

FIG. 2. FREQUENCY OF OBSERVATIONS 0, 1, 2, ETC. WITH VARIOUS n for *S. stricta*, AS IN TABLE VI.

TABLE V. OBSERVED FREQUENCY OF *Lespedeza capitata* (THOMSON 1952) AND CONTAGIOUS DISTRIBUTIONS.

Plants	Obs.	$n = 0$*	$n \to \infty$	Max. Lik.* $n = 0$
0	7178	7265.1	7217.6	7188.4
1	286	134.0	218.6	219.6
2	93	118.9	105.5	140.6
3	40	71.1	50.9	61.6
4	24	32.5	24.5	21.1
5	7	12.3	11.8	6.2
6	5	4.2	5.7	1.7
7	1	1.3	2.8	.5
8	2	.4	1.3	.1
9	1	.1	.6	.0
10	2	.0	.3	.0
11	1	.0	.2	.0
12+	0	.0	.2	.0

*As calculated by Thomson.

tribution only slightly different from the case of $n = 0$. In studies in the field it might be seriously considered whether Thomas' distribution cannot be bettered by some member of the family of contagious distributions.

The distributions for $n = 0$ and 5 and for $n \to \infty$, in the case of *S. stricta*, are shown in Fig. 2, because they illustrate rather boldly the change characteristic of increasing n. As in Fig. 1 for $n = 0$ there is bimodality which finally is replaced by a unimodal distribution. It is worthy of note, that in Fig. 2, the bimodality persists so late as $n = 5$.

In contrast to the experience of Archibald, in that of Upholt and Craig (1940) contagious distributions failed to fit data on black scale (insects). The difficulty is apparently similar to that reported in Table III on *L. decemlineata* in that the actual machinery of dispersion of the insects is not close enough to that supposed fundamentally to exist. In the case of *L. decemlineata*, the failure was probably due to the superimposition of larvae of various age with various distribution. In the case of the black scales, according to Upholt and Craig, the failure is probably due to the fact that one is dealing not with the distribution of daughters arising from an initial random dispersion of mother scales but with granddaughters, dispersed in various numbers from daughter scales themselves in a contagious distribution, and so on for several generations. Present endeavors support Upholt and Craig in failing to find a suitable contagious distribution; as can be seen in Table VII

TABLE VI. OBSERVED FREQUENCY OF *S. stricta* (ARCHIBALD 19 48) AND CONTAGIOUS DISTRIBUTION WITH VARIOUS n.

Plants	Obs.	$n = 0$	$n = 5$	$n \to \infty$
0	4	10.4	6.9	6.0
1	3	4.0	6.7	6.9
2	8	6.6	7.7	8.0
3	13	7.9	8.2	8.5
4	11	8.2	8.3	8.6
5	9	8.0	8.1	8.3
6	8	7.6	7.7	7.8
7	10	7.1	7.0	7.1
8	3	6.4	6.3	6.3
9	3	5.7	5.5	5.5
10	8	5.0	4.8	4.7
11	3	4.2	4.1	4.0
12	4	3.5	3.4	3.3
13	4	2.9	2.8	2.7
14	0	2.4	2.3	2.2
15	3	1.9	1.9	1.8
16	0	1.5	1.5	1.4
17	0	1.2	1.2	1.1
18	1	.9	1.0	.9
19+	3	2.6	2.6	2.9
χ^2		18.67	17.20	15.32
$P\chi^2$		.18	.25	.36

where the fit for $n = 2$ for $n \to \infty$ are shown but no correspondence with the empirical distribution appears.

The data of Marshall (1936) on frequency per plant of larvae of the American bollworm, *Heliothis obsoleta* F. on maize may be of interest because it has attracted considerable attention and because there exists a possibility that it is contagious. As explained by Marshall, the moth lays eggs singly. There seems, to the present authors, to exist, however, a possibility that a moth selects a plant randomly and then lays several eggs on that plant before moving on to another plant and that this group of eggs is somewhat analogous to the egg mass of the corn borer. Then we may expect there to be a random selection of sites on each of which a random number of eggs is laid. If such is the situation a contagious distribution should result. For these data, as in Table V, there is apparent no reason why any other value of n will better $n \to \infty$. For this n the value of χ^2 is better than that for $n = 0$,

TABLE VII. OBSERVED FREQUENCY OF BLACK SCALES (UPHOLT AND CRAIG 1940) AND TWO CONTAGIOUS DISTRIBUTIONS.

Scales	Obs.	$n = 2$*	$n \to \infty$
0	199	497.1	436.4
1	124	16.0	31.4
2	106	8.0	28.9
3	65	5.2	26.7
4	42	3.8	25.0
5+	285	290.9	272.6

*As calculated by Upholt and Craig.

TABLE VIII. OBSERVED FREQUENCY OF *H. obsoleta* (MARSHALL 1936) AND FIRST TWO CONTAGIOUS DISTRIBUTIONS AND THEN THE GREENWOOD-YULE DISTRIBUTION

Insects	Obs.	$n = 0$	$n \to \infty$	Greenwood-Yule Dist.*
0	206	267.3	226.2	200.4
1	143	90.5	140.7	165.8
2	128	105.3	113.9	123.8
3	107	91.8	87.7	89.1
4	71	70.3	65.0	62.9
5	36	51.0	46.9	43.9
6	32	36.0	33.0	30.4
7	17	24.8	22.9	20.9
8	14	16.5	15.6	14.3
9	7	10.8	10.4	9.8
10	7	6.9	6.9	6.7
11	2	4.3	4.6	4.5
12	3	2.6	3.0	3.1
13	3	1.6	1.9	2.1
14	1	.9	1.2	1.4
15	1	.6	.8	1.0
16	1	.3	.5	1.9
17	2	.2	.3	
18	1	.1	.2	
19+	0	.2	.3	
χ^2		68.92	17.99	13.58
$P\chi^2$		.000	.082	.257

*As calculated by Walker (1942).

but not good. These data are apparently not in a contagious distribution. On Marshall's data, Walker (1942) made a study, from which we should note particularly the fit of the Greenwood-Yule distribution in Table VIII, which is fairly good. This distribution supposes that the larvae occur randomly in a small locality but that their probability varies over the field in a Pearson Type III distribution.

In final illustration of the use of contagious distributions with high n it may be interesting to consider, as in Table IX, the data on yeast

TABLE IX. OBSERVED FREQUENCY OF YEAST CELLS (NEYMAN 1939) AND TWO CONTAGIOUS DISTRIBUTIONS.

Cells	Obs.	$n = 0$*	$n \to \infty$
0	213	214.8	214.4
1	128	121.3	122.1
2	37	45.7	45.3
3	18	13.7	13.5
4	3	3.6	3.5
5	1	.8	.8
6+	0	.1	.4

*As calculated by Neyman.

due to "Student" (1907) in Neyman's (1939) original paper. He found that $n = 0$ fitted well but as can be seen $n \to \infty$ is at least as apt. This case is curious because it seems immaterial what value is given n.

To conclude the present section we may make some observations on those cases where the contagious distributions might have been expected to apply and did so in fact, by the judicious choice of n. The similarity of the tails for all n indicates perhaps that our attention should be focused upon the first few members of the distribution. Among these members, P_0 is of transcendent importance. For $n = 0$ the theoretical value may be manifestly too great, whence we are advised by (40) to try $n > 0$, since we know this theoretical member will always diminish with increase in n. The behavior of P_0 for various n may be judged more fully from the various preceding tables. The value of P_1 may also be profitably considered since it seems generally to be greater for $n = 1$ than for $n = 0$ (and particularly greater if P_0 is much greater than P_1) and this is another indication of the proper value of n. Our approach is similar to that of Thomas (1949), who fitted by making theoretical and observed class frequencies agree. We, however, are

inclined to do so only in connection with the estimation of n, while we should still get m_1 and m_2 by the method of moments.

We may further make some general observations, on the goodness of fit for various values of n. From a fairly wide experience it seems that χ^2 changes very rapidly when n is small but increasingly slowly as n becomes great. Thus in Table II, $n = 8$ and $n \to \infty$ gave us 6.23 and 6.21, respectively, for values of χ^2. Hence if a high n is called for, we may have an unlimited choice of the n to be used. Since the case of $n \to \infty$ is the easiest to calculate, it would be chosen.

6. *The difficulty of explicit determination of n*

From the results of Section 5, it appears that contagious distributions may be fitted to many data, but there is perhaps a question as to the appropriate n. Some way should be found to get it empirically. The difficulty is the same as in Neyman's original paper where there was no clear indication as to which of the three Types A, B or C, i.e., $n = 0$, 1 or 2, should be chosen. The writers have considered the possibility of getting n by the method of moments (as for m_1 and m_2) but the outcome has been unhappy.

Let us first consider the estimation of m_1 , m_2 and n simultaneously from (35) and (36), for the data on *P. nubilalis*, in 1936, of Table I. We find $\mu_1' = 2.982,142,857$, $\mu_2 = 14.946,109,69$ and $\mu_3 = 42.619,254,54$ so that we estimate $n = -1.95$ ($m_1 = -25.61$ and $m_2 = +.11$). It should be noted that n is negative. In violent contrast, Table II suggests that, at least from the point of view of getting a minimal χ^2, under the condition that m_1 and m_2 yield the first two empirical moments, n should be about 4. For the data on *P. nubilalis*, in 1937, the moments are $\mu_1' = .821,428,571,4$, $\mu_2 = 2.182,397,959$ and $\mu_3 = 10.746,264,58$ and on estimating by the method of moments we get $n = -4.38$ ($m_1 = 1.41$ and $m_2 = -1.97$), again negative. Similarly for *P. nubilalis*, of 1938, of the same series, we find from the empirical moments, $n = +.24$ which we would call too small, since it is close to Neyman's Type A which Beall (1940) found inferior to Type C. So we may say that again n is erring in the negative direction.

Let us consider some more data on *P. nubilalis* Hubn., from Beall (1940, Table II, Treatment 1). From the empirical moments in (62), $n = 1.95$. It appears however, that following our procedure in Table II, etc., some more extreme case ($n > 2$) would give a better fit. If such is the case our estimate of n is low. Similarly for the data on *P. nubilalis*, of Beall (1940, Table II, Treatment 4), $n = -4.40$. The method of moments again seems no wise satisfactory.

Generally speaking, the foregoing work suggests that (36) with empirical moments, substituted, gives estimates of n too small and certainly bad. There is, of course, little reason why values of the parameters, so obtained, should give very good correspondence between the theoretical and empirical distributions, although the method does work in some connections. Further, it would not seem that the third moment could be highly sensitive to changes in n. This can be seen for instance from Table II where there is a fairly extensive exploration of the variation in the contagious distributions as n varies. Obviously the third moment varies little with change in n, i.e., the variation is mostly in the values of P_0 , P_1 and a few early members while the tail of the distribution is little changed. Such has tended to be our experience with data generally. Under these circumstances (36) becomes very sensitive to chance fluctuations and n becomes more or less random. The suggestion that n seems to be underestimated seems hardly worthy of further exploration.

It seems that in practice one should choose as in Section 5, the value of n from a consideration of the proportion of cases in the first class, i.e., the proportion of observations 0, and then find m_1 and m_2 by the method of moments. In the use of the proportion of cases in the first class, our procedure is not unlike that of Thomas (1949) who used this as one consideration for finding the values of m_1 and m_2 .

7. *Illustrative application of procedure*

In order to make abundantly clear the actual procedure of fitting the generalized contagious distributions, with any value of n, there follow a few simple illustrations. The data are those of Table II, on *P. nubilalis* for 1936. The principal illustration is that of fitting for $n = 2$ (Type C).

In the first place it should be clearly realized that while the theory rests on certain characteristic functions, in particular on Equ. (2), and on the associated generating functions and while these involve awkward operations of differentiation these are not employed in the routine of calculation. This is done by the use of certain recurrent functions, as in Section 3. We shall consider the procedure hinging on Equ. (22) and previously designated as that commonly recommended. The total operation, discussed in great detail below, is directed toward the calculation of the successive probabilities P_r . In order to obtain these we must first calculate the numerical coefficients F_k . Generally, however, these coefficients must themselves be calculated from an antecedent recurrent operation.

We must first estimate the values m_1 and m_2 from Equ. (35). If, for example, we choose $n = 2$, we have accordingly

$$\left.\begin{aligned} m_2 &= 2\frac{(\mu_2 - \mu_1')}{\mu_1'} = 2\frac{14.946,109,693 - 2.982,142,857}{2.982,142,857} \\ &= 8.023,738,236 \\ m_1 &= \frac{3\mu_1'}{m_2} = \frac{3(2.982,142,857)}{8.023,738,236} = 1.114,995,069 \end{aligned}\right\} \quad (59)$$

Parenthetically, we may note that the second moment should be calculated by the formula

$$\mu_2 = \frac{1}{N}\sum_{N}(X - \bar{X})^2 \quad (60)$$

i.e., the divisor is N not $N - 1$ as is done in estimating standard deviations in certain connections. Note also in all these calculations it is necessary to use a surprisingly large number of places.

Towards the recurrent coefficients F_k find, repeating (20),

$$f(0) = n!(-m_2)^{-n}\left\{e^{-m_2} - \sum_{s=0}^{n-1}\frac{(-m_2)^s}{s!}\right\} \quad (61)$$

For the illustrative case of $n = 2$,

$$\begin{aligned} f(0) &= 2(-8.023,738,236)^{-2}(e^{-8.023,738,236} - 1 + 8.023,738,236) \\ &= .218,205,184,4 \end{aligned} \quad (62)$$

The second step is to calculate from (21) and (16)

$$\begin{aligned} F_0 &= (m_2 + n)f(0) - n \\ &= (10.023,738,236)(.218,205,184,4) - 2 \\ &= .187,231,651 \end{aligned} \quad (63)$$

The third step is to calculate

$$\begin{aligned} F_1 &= \{(m_2 + n)^2 + n\}f(0) - n\{(m_2 + n) + 1\} \\ &= .313,171,436 \end{aligned} \quad (64)$$

Now using these two first values, it is necessary to compute for all higher P_r the comparatively simple Table X. Numerically, the procedure is as in Table XI. Column (2), for a given k, is the product of a value in Column (1) and the value of m_1 , F in the preceding row all divided by k, i.e., the value in column (2) when $k = 3$ is [(13.023,738,236) (.424, 197,564)/3] = 1.841,546,010. Column (3), for a given k, is the product of m_2 and a value of m_1F in the second preceding row divided by $k - 1$,

TABLE X. THE CALCULATION OF RECURRENT COEFFICIENTS FOR THE GENERAL CASE.

k	$m_2 + n + k$	$\frac{(m_2 + n + k)}{k} m_1F_{k-1}$	$\frac{-m_2}{k-1} m_1F_{k-2}$	m_1F_k
0	$m_2 + n$	—	—	m_1F_0
1	$m_2 + n + 1$	—	—	m_1F_1
2	$m_2 + n + 2$	$\frac{m_2 + n + 2}{2} m_1F_1$	$\frac{-m_2}{1} m_1F_0$	m_1F_2
3	$m_2 + n + 3$	$\frac{m_2 + n + 3}{3} m_1F_2$	$\frac{-m_2}{2} m_1F_1$	m_1F_3
. .	. . . . .	. . . . .	. . . . .	. . . .

TABLE XI. NUMERICAL ILLUSTRATION FOR *P. nubilalis* 1936 OF GETTING THE RECURRENT COEFFICIENTS, FOR $n = 2$.

k	(1) $m_2 + 2 + k$	(2) $\frac{m_2 + 2 + k}{k} \times m_1F_{k-1}$	(3) $\frac{-m_2}{k-1} m_1F_{k-2}$	(4) m_1F_k
0	—	—	—	.208,762,368
1	—	—	—	.349,184,607
2	12.023,738,24	2.099,252,155	−1.675,054,591	.424,197,564
3	13.023,738,24	1.841,546,010	−1.400,882,941	.440,663,069
4	14.023,738,24	1.544,935,884	−1.134,550,071	.410,385,813
5	15.023,738,24	1.233,105,807	− .883,941,280	.349,164,527
6	16.023,738,24	.932,486,774	− .658,565,668	.273,921,106
7	17.023,738,24	.666,166,023	− .466,934,127	.199,231,896
8	18.023,738,24	.448,862,943	− .313,981,672	.134,881,271
9	19.023,738,24	.285,105,111	− .199,823,073	.085,282,038
10	20.023,738,24	.170,766,520	− .120,250,223	.050,516,297
11	21.023,738,24	.096,549,218	− .068,428,075	.028,121,143
12	22.023,738,24	.051,611,059	− .036,848,140	.014,762,919
13	23.023,738,24	.026,145,968	− .018,803,058	.007,342,910
14	24.023,738,24	.012,600,297	− .009,111,831	.003,488,466
. . . .	. . . . .	. . . . .	. . . . .	. . . . .

i.e., the value in column (3) when $k = 3$ is $[(-8.023,738,236)(.349,184,607)/2] = -1.400,882,941$. Column (4) is the sum of the items of the same line but in columns (2) and (3).

In order to get finally the probabilities P_r for the case of $n = 2$ from (14)

$$\begin{aligned} P_0 &= \exp\{m_1\{f_n(0) - 1\}\} \\ &= \exp\{1.114,995,069(.218,205,184,4 - 1)\} \\ &= .418,24 \end{aligned} \tag{65}$$

Then all the succeeding values P_1 , P_2 , etc., are calculated, as in Table XII when the m_1F values are those proper for $n = 2$, as from Table XI.

TABLE XII. THE CALCULATION OF SUCCESSIVE PROBABILITIES P_r FOR ANY VALUE OF n.

r	P_r
0	P_0 as in Equ. (65)
1	$P_1 = (m_1F_0)P_0$
2	$P_2 = \{(m_1F_0)P_1 + (m_1F_1)P_0\}/2$
3	$P_3 = \{(m_1F_0)P_2 + (m_1F_1)P_1 + (m_1F_2)P_0\}/3$
4	$P_4 = \{(m_1F_0)P_3 + (m_1F_1)P_2 + (m_1F_2)P_3 + (m_1F_3)P_0\}/4$
. . . .	. . . . .

TABLE XIII. NUMERICAL ILLUSTRATION FOR *P. nubilalis* OF GETTING FOR $n = 2$ THE SUCCESSIVE PROBABILITIES, P_r.

r	P_r
0	.418,24
1	.087,31 = (.208,762)(.418,24)
2	.082,14 = {(.349,185)(.418,24) + (.208,762)(.087,31)}/2
3	.075,02 = {(.424,198)(.418,24) + (.349,185)(.087,31)
4	.066,42 { (.208,762)(.082,14)}/3
5	.057,00
6	.047,50 etc.
7	.038,55
8	.030,60
9	.023,88
10	.018,39
11	.014,03 etc.
12	.010,63
13	.008,02
14	.006,01
. . . .	. . . . .

In practice it is convenient to rewrite those coefficients so that the last becomes first and the first last and then apply the inverted series m_1F_k to the column of P_r as it develops. To get each new value of P_r the inverted column is then slid down one step. The results are in Table XIII.

For any other value of n, excepting $n = 0$ and $n \to \infty$, the recurrent coefficients are calculated in essentially the same way as for $n = 2$. In Equ. (59), (60), (61), (63) and (64) and in Table X, the appropriate value of n must be used. The remaining references for P etc. are identical with those for the case of $n = 2$.

The case of $n = 0$ is peculiar in that the recurrent coefficients F_k, can be found rather simply. Note that in general from (16) and (18),

$$F_k = \frac{m_2^{k+1}e^{-m_2}}{k!} \tag{66}$$

Then

$$F_0 = m_2e^{-m_2} \tag{67}$$

and $F_1 = m_2F_0$. The subsequent, successive values of F_2, F_3, etc., may be found very simply on the general principle illustrated by the operation, $F_2 = m_2F_1/2$, $F_3 = m_2F_2/3$, etc. Then the successive values of P_r should be calculated as in Table XII, employing from (14),

$$P_0 = \exp\{-m_1\} \exp\{m_1e^{-m_2}\} \tag{68}$$

The calculation for the case of $n \to \infty$ is numerically, particularly simple. There is no value m_2, but rather the matter can be most conveniently arranged about the numbers:

$$c = \frac{\mu_2 - \mu_1'}{\mu_1'} = 4.011,869,118 \tag{69}$$

and

$$\frac{c}{2+c} = \frac{4.011,869,118}{6.011,869,118} = .667,324,760,3 \tag{70}$$

and also

$$m_1 = \frac{2\mu_1'}{c} = 1.486,660,092 \tag{71}$$

The calculation of the values m_1F_k is very simple. First we calculate

$$m_1F_0 = \frac{4\mu_1'}{(2+c)^2}$$

and then successively,

$$m_1F_1 = \frac{2c}{2+c}\, m_1F_0\,, \qquad m_1F_2 = \frac{3}{2}\frac{c}{2+c}\, m_1F_1\,,$$

$$m_1F_3 = \frac{4}{3}\frac{c}{2+c}\, m_1F_2\,, \qquad \text{etc.}$$

For the numerical illustration of *P. nubilalis*, 1936, of Table II,

$$\left.\begin{aligned} m_1F_0 &= .330{,}04 \\ m_1F_1 &= .440{,}49 \\ m_1F_2 &= .440{,}93 \\ m_1F_3 &= .392{,}32 \end{aligned}\right\} \tag{72}$$

Finally, by direct calculation,

$$P_0 = \exp\left\{-\frac{2\mu_1'}{2+c}\right\} \tag{73}$$

The other P_r values, i.e., P_1 , P_2 , P_3 , etc., are again calculated from Table XII.

It cannot be denied that the calculation of contagious distributions is a little involved, although the difficulties are minimal for $n = 0$ and $n \to \infty$. There has appeared no practical solution by way of the formation of facilitating tables. Such has been attempted by Thomson (1952) for the case of $n = 0$ but little seems to have been gained in this, one of the two most simple cases.

8. *Summary and discussion*

Neyman (1939) proposed a class of contagious distributions applicable to situations where individuals or items are supposed initially dispersed in randomly scattered groups—egg masses in the case of insects or clumps in the case of bacteria—which are subject to a chance graduation in size. We have obtained a generalized contagious distribution, characterized by a critical parameter which we call $0 \leq n \leq \infty$, and not necessarily an integer. Neyman's original types are cases $n = 0$, 1 or 2.

There existed a considerable problem in calculating the generalized contagious distributions, which the senior author had previously solved in part by the development of a recurrent method for finding the probabilities P_r of successive observations 0, 1, 2 $\cdots$ r $\cdots$. This is discussed by Neyman (1939:47). While this approach made it physically possible to get the distributions the work remained heavy, except for

$n = 0$. The problem is that the recurrent coefficients may themselves be difficult to find. The writers, accordingly, have developed two schemes for finding these coefficients by secondary recurrent methods. One of these methods is particularly simple in that only two previous members of a series of coefficients are needed to calculate the next. They have also developed a scheme for building up the coefficients by a method of differences, for n an integer, which is particularly accurate and useful in some connections. These methods make it almost as easy to calculate a contagious distribution as a binomial.

The form of the generalization as $n \to \infty$ is naturally of interest. It resembles somewhat a negative binomial, although not identical. Very frequently it fits data very well. In the cases we have examined n increasing beyond say 10 makes very little difference and perhaps the case for $n \to \infty$ may often be used, particularly, since it is very easily calculated.

Alternate forms (n varying) of generalized distribution were tried against various empirical distributions. The value of n was chosen arbitrarily and the other parameters were found by the method of moments. The various examples were chosen from the literature; in particular cases, where $n = 0$ had been found to fit poorly, were considered. Cases exist where $n = 0$ gave the best fit, although from our choice of material this class was ill explored. There was found only one case where $0 < n < \infty$ gave the best fit—theoretically this class may be fairly frequent. There were several cases where $n \to \infty$ gave the best fit. There was one case where the magnitude of n seemed immaterial. In some cases where quite a good fit had been obtained with $n = 0$, it was bettered by $n > 0$. In two cases where the known details of insect distribution in no wise corresponded to those presupposed in a Neyman type distribution (random distribution of groups of random magnitude) the fit was bad.

There exists a considerable problem as to the best means of deciding the value of n, characterizing the type of distribution. The method of moments seems *a priori* and *a posteriori* hopeless. The best method seems to be that of setting n such that P_0, the proportion of zero observations, agrees with the empirical result while the other two parameters m_1 and m_2 are chosen so that the theoretical and empirical moments agree.

The present study is necessarily restricted to the defined purpose of making a fairly obvious extension of the class of contagious distributions proposed by Neyman (1939). This has been accomplished and the generalized family, not unexpectedly, can better represent empirical data than the former part of the family. The utility of the generaliza-

tion, in dealing with biological data will be the same as that of Neyman's previous types and this he has discussed at considerable length in the concluding remarks of his original paper of 1939.

The present results will permit explorations to be made for the existence of phenomena that may be graduated by contagious distributions. This may be useful in trying to determine the machinery of dispersion. Experience with contagious distributions may also be useful in the analysis of experimental results; such was the intention of the whole field of investigation in the first place. It may be noted that the transformation of Beall (1942) from observations x to

$$x' = q^{-1/2} \sinh^{-1} (qx)^{1/2} \tag{74}$$

may have some applicability to contagious distributions since it is based on a presumption that

$$\mu_2 = \mu_1' + q\mu_1'^2 \tag{75}$$

where q is some constant and this situation exists for the contagious distributions, as for the negative binomial. It is, however, probable that the transformation is not so apt for the contagious distribution as for the negative binomial because although in the former case the standard deviation may be made independent of the mean it can hardly be supposed that, in general, the distribution is normalized, as seems to be approximately the case for the binomial. The whole problem and the role, however, of such transformations is very ill understood and we cannot be final.

The writers are beholden to Professor Jerzy Neyman for his advice at the beginning of the present work.

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Appendix—An alternate generalization of the contagious distributions.

The generalization (2) of Neyman's contagious distributions is fairly obviously suggested by the form of Equ. (1); it may however be also shown to arise by an extension of Neyman's operations in setting up his types A, B and C. We may start with the general characteristic function of a contagious distribution in the form of Neyman's Equ. (26),

$$\log \phi(t) = m\lambda(e^{it} - 1) + Am \sum_{n=2}^{\infty} \frac{\lambda^n (e^{it} - 1)^n}{n!} P_n \qquad (76)$$

where P_n are moments of a random variable Z {which Neyman has identified with his P (ξ, η)} the probability that a given individual caterpillar etc., will be found within an experimental plot. There is an important condition that always $P_1 = A^{-1}$. Neyman then introduces the function $F(z)$ where z is any number between zero and unity such that $F(z)$ will possess all the properties of the integral probability law of Z.

Neyman has presupposed certain conditions concerning $p(z) = dF/dz$: first, that it exists and secondly that it is either zero or a function of z (for Type A, Z is a constant, A^{-1}). Then, as various approximations to F, he writes:

$$\left.\begin{aligned}
&\text{for Type A } p_0(z) \equiv 0 \text{ for } 0 < z < A^{-1},\ A \geq 1 \\
&\text{for Type B } p_1(z) = \tfrac{1}{2}A \text{ for } 0 < z < 2A^{-1},\ A \geq 2 \\
&\qquad\qquad = 0, \text{ elsewhere} \\
&\text{for Type C } p_2(z) = \frac{2A^2}{9}(3A^{-1} - z) \text{ for } 0 < z < 3A^{-1},\ A \geq 3 \\
&\qquad\qquad = 0, \text{ elsewhere}
\end{aligned}\right\} \qquad (77)$$

Equ. (77) suggests that one can write, generally,

$$\left.\begin{aligned} p_r(z) &= \frac{rA}{(r+1)^r}\{(r+1)A^{-1} - z\}^{r-1} \\ 0 &< z < (r+1)A^{-1},\ A \geq r+1 \\ p_r(z) &= 0, \text{ elsewhere} \end{aligned}\right\} \tag{78}$$

For illustration consider

$$\begin{aligned} p_3(z) &= \frac{3A^3}{64}(16A^{-2} - 8A^{-1}z + z^2) \text{ for } 0 < z < 4A^{-1},\ A \geq 4 \\ &= 0, \text{ elsewhere} \end{aligned} \tag{79}$$

The form of (78) satisfies the two requirements on $F(z)$.

The moments of $p_r(z)$, as from (78), are

$${}_rP_n = \frac{n!r!\{(r+1)A^{-1}\}^n}{(n+r)!} \tag{80}$$

whence on substitution of (80) in (76) we get the general characteristic function

$$\begin{aligned} \log \phi(t) &= m\lambda(e^{it} - 1) + Am \sum_{n=2}^{\infty} \frac{\lambda^n(e^{it} - 1)^n r!\{(r+1)A^{-1}\}^n}{(n+r)!} \\ &= -m_1 + r!m_1 \sum_{n=0}^{\infty} \frac{m_2^n(e^{it} - 1)^n}{(n+r)!} \end{aligned} \tag{81}$$

where $m_1 = Am$ and $m_2 = [(r+1)\lambda]/A$. Equ. (81) is identical with the general characteristic function that we assumed in the beginning of the paper, i.e., (2).

QUALITY × QUANTITY INTERACTION

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INTRODUCTION

Fisher (4), in a reply to a query to this journal, has discussed the estimation of the effect of quality and of the quality × quantity interaction in the case of an experiment with 4 qualities of a fertiliser at 3 equally spaced levels, the lowest of which is not zero.

As the originator of this query, my object was to obtain information concerning the general case of a situation common in experimentation. Textbooks are not very illuminating, since they all deal with the same special case, that of a number of qualities at 3 equally spaced levels, the lowest of which is zero, so that dummy treatments occur at the zero level. Thus Fisher (3) shows that the ordinary subdivision (based on a hypothesis of independent additive effects of the factors) of the sum of squares of such an interaction table into sums of squares for main effects, dummy effects, and interaction is unsatisfactory, and proposes the more natural hypothesis that quality differences are proportional to quantity applied instead of constant for all quantities. He describes his estimation of quality effects as "calculating the 'regression' of the manurial response upon the manurial difference to which it is for the present purpose to be considered as proportional". An alternative orthogonal subdivision of the sums of squares for quality and for interaction of quality and quantity (as obtained in an ordinary interaction table) is based on the identity

$$\tfrac{1}{2}(x + y)^2 + \tfrac{1}{2}(x - y)^2 = \tfrac{1}{5}(2x + y)^2 + \tfrac{1}{5}(x - 2y)^2,$$

where the left hand side of the identity represents the ordinary subdivision and the right hand side the subdivision proposed by Fisher. From the first term on the right hand side is derived the sum of squares for quality on the hypothesis of proportional response. The residue, which would be zero if the response to quality was exactly proportional to quantity (since $x - 2y$ would be constant for each quality), is labelled "interaction" of quality and quantity. The tendency might be to regard the significance of this interaction as demonstrating the

fact that "the qualities are varying in their effect at different levels". A little thought, however, shows that such variation, if present, is actually an effect of quality itself. As Fisher puts it: "The quantitative contrasts are differences caused by quantitative variations in the very substances which the qualitative comparisons are intended to compare". The ordinary type of statement used in interpreting an interaction effect is therefore not applicable. Later (p. 148) Fisher supplies the type of interpretation necessary. Fisher also mentions the applicability of his method of subdivision to the case when the ratio of the two quantities used is different from 1:2. Yates (7) restates Fisher's findings without discussion, except to show how the dummy treatments may be used to eliminate block differences in calculating the sums of squares for the interaction of quality and quality $\times$ quantity with the third factor of a $3 \times 3 \times 3$ confounded design. Cochran and Cox (2) discuss the subdivision of the sum of squares for an identical type of interaction table on the same hypothesis. The estimate of quality effects is shown to be actually a weighted mean. They also derive Fisher's interaction of quality and quantity as "differences in curvature". The effect of quality is labelled "differences in linear response". This change of nomenclature has the effect of making it easier to see how the analysis could be carried out if there were more than two quantitative levels in addition to the zero level. Nevertheless none of the above accounts does actually indicate how the methods are to be extended when the number of non-zero levels exceeds two, or when there is no zero level.

Kempthorne (5), under the heading "Partially factorial experiments", discusses a 3×3 Quality $\times$ quantity table, the quantities being equally spaced. He states that, if none of the amounts is zero, there is no new difficulty. Taking the usual example, with the lowest level zero, he shows how to fit three quadratic response curves with common base point, there being no restrictions on the seven constants fitted. He also discusses a model in which the basic response law is $\mu + fx + gx^2$, the qualities differing in containing different concentrations of the basic causative factor, so that an amount x of the first quality is considered equivalent to quantities λx and τx of the second and third qualities. It is noted that, when the response law is assumed to be linear, the situation corresponds to that described by Yates (and Fisher). However, certain difficulties with this model are mentioned, and it is concluded that "satisfactory methods for the analysis of such data have not been evolved for more or less general situations".

Williams (6) discusses a more general problem, proposing a model

in which the effects of one factor (quality) are considered to be proportional at different levels of the other factor (quantity). The proportions are, however, not simply those of the quantities applied. Williams states that the simpler hypothesis, if realistic, is to be preferred, but that in many experiments involving qualities and quantities the effects will not be proportional to quantity but will increase less rapidly than quantity. His proposed method of analysis therefore includes the estimation of these proportions from the data in such a way that the sum of squares for the weighted main effects of qualities (c.f. Cochran and Cox) is a maximum and the residual interaction sum of squares a minimum. However, since the method includes the finding of latent roots of a matrix, it is not likely to commend itself to the average experimentalist who so often must perforce do his own analyses.

The more complex type of analysis such as that proposed by Williams seems to be more suitable when it is the nature of the response curves of the different qualities which is the main subject of investigation. In practice it will more often be the case that the experimentalist merely wishes to estimate the yields of his various treatments, comparisons being made by difference, supported by statistical tests of significance if possible. As Kempthorne points out, this can always be done irrespective of any knowledge of the response curves, whether assumed *a priori* or presumed from the data. Suitable subdivision of the treatments set of degrees of freedom will, however, enable the nature of the treatment differences to be more clearly brought out. Even the ordinary additive hypothesis will achieve this to some extent, for, if the interaction proves to be significant, then it will be realised that main effects based on this hypothesis are of little value and attention is directed back to individual treatment means. On the other hand the experimentalist is then at liberty to try some other hypothesis (the simpler the better) which may explain the nature of the observed effects. Fisher, Yates, and Cochran and Cox therefore test the hypothesis that the response to quality is proportional to quantity applied as being more natural than the hypothesis of equal additive effects in these circumstances. When these simple hypotheses have been exhausted, there is certainly no compulsion to undertake any more complex analysis such as those suggested by Kempthorne or Williams. Furthermore there is no doubt that an analysis based on the proportional hypothesis, whether realistic or not, provides the answer to very realistic questions, viz:—(1) "What is the average response to each unit application of each quality of fertiliser? (2) Does this average response vary from quality to quality? (3) Is the response per unit application of a quality

consistent over the range of applications of that quality?". These questions are in general more realistic than those posed under the additive hypothesis, viz:—(1) "What is the average yield of each quality over its various applications? (2) Do these averages differ? (3) Are differences between qualities constant over the different applications?" It would seem to me that, provided one is not interested in the exact nature of the response curves, the answers to the first three questions, coupled with an appropriate examination of the interaction table, will provide an adequate routine analysis in this situation.

The subject of this paper concerns, therefore, only the analysis of quality $\times$ quantity interaction tables when the proportional hypothesis is adopted. Although Fisher (4), while stating that opinions may legitimately differ, has demonstrated the method to be followed in a particular case, it is possible that the ordinary experimentalist who does not claim to be a professional biometrician may be glad to have a little more meat to chew on than the rather bare bones of an algebraic identity! The statistical situation is actually identical with that known in biological assay as a "slope-ratio assay", as described, for example, by Bliss (1), except that there the zero dose is usually represented. There is, however, a difference in experimental objective, and in addition many workers familiar with the analysis of field experiments may be unacquainted with the literature of biological assay.

STATISTICAL MODEL FOR AN ORDINARY INTERACTION TABLE

Suppose we consider the case of 3 quantities ($x = 1, 2, 3$), none of which is zero, and for simplicity the case of 2 qualities only. This latter simplification does not impair the generality of what follows except in respect of minor details. The yields are set out in an interaction table as under, and without loss of generality we shall treat them as derived from a single replication.

Qualities	Quantities			Totals
	1	2	3	
A	y_{11}	y_{12}	y_{13}	Y_{10}
B	y_{21}	y_{22}	y_{23}	Y_{20}
Totals	Y_{01}	Y_{02}	Y_{03}	Y
Differences	δy_1	δy_2	δy_3	

The ordinary procedure for partitioning the sum of squares for this interaction table may be summarised as

$$\underset{\text{TOTAL S.S.}}{\sum_i \sum_j y_{ij}^2 - \tfrac{1}{6}Y^2} = \underset{\text{QUALITIES}}{(\tfrac{1}{3}\sum_i Y_{i0}^2 - \tfrac{1}{6}Y^2)} + \underset{\text{QUANTITIES}}{(\tfrac{1}{2}\sum_j Y_{0j}^2 - \tfrac{1}{6}Y^2)}$$

$$+ \underset{\text{INTERACTION}}{(\sum_i \sum_j y_{ij}^2 - \tfrac{1}{3}\sum_i Y_{i0}^2 - \tfrac{1}{2}\sum_j Y_{0j}^2 + \tfrac{1}{6}Y^2)}$$

This partitioning implies the assumption that with no interaction the differences δy_j would be the same apart from random fluctuations.

In practice the sum of squares for quantities will usually be subdivided into linear and quadratic effects, with corresponding subdivision of the interaction sum of squares. If we assume for the meantime that responses are purely linear, the subdivision is equivalent to postulation of the following model (ignoring random components),

Qualities	Quantities			
	1	2	3	
A	$m_1 - b_1$	m_1	$m_1 + b_1$	(Model 1)
B	$m_2 - b_2$	m_2	$m_2 + b_2$	

where m_1 and m_2 , b_1 and b_2 represent the mean levels of yield and the linear regression coefficients, respectively, for the 2 qualities. We may replace m_1 and m_2 by $m - q$ and $m + q$ indicating deviations from a common mean level brought about by the effect of quality. Similarly, we may replace b_1 and b_2 by $b - \delta b$ and $b + \delta b$, where b indicates a common regression coefficient and δb is a correction (or interaction component) to allow for the possibility that b_1 and b_2 may differ. The model now is:

Qualities	Quantities			Means	
	1	2	3		
A	$m - q - (b - \delta b)$	$m - q$	$m - q + (b - \delta b)$	$m - q$	(Model 2)
B	$m + q - (b + \delta b)$	$m + q$	$m + q + (b + \delta b)$	$m + q$	
Means	$m - b$	m	$m + b$	m	
Differences	$2q - 2\delta b$	$2q$	$2q + 2\delta b$	$2q$	

Thus m and q are estimated from the mean and mean difference, respectively, while b and δb may be estimated by applying the appropriate orthogonal polynomial values $(-1, 0, 1)$ to the means and differences respectively, for the 3 quantities. The procedure is seen to be equivalent to fitting individual linear regression lines to the data supplied by each of the 2 qualities at 3 levels and to fitting a common regression line to the data for both qualities combined. The sums of squares corresponding to b and δb represent, respectively, the sum of squares due to fitting the common regression line and a sum of squares representing deviations of the slopes of the individual regression lines from that of the common regression line.

STATISTICAL MODEL ON THE ASSUMPTION OF PROPORTIONAL RESPONSE

In Model (2), if $\delta b = 0$ (i.e. if there is no interaction), the differences between qualities for the 3 levels are all equal to $2q$, so that the effect of the quality difference is assumed the same for all levels of the fertiliser. However, Fisher (3) has pointed out that a more natural hypothesis would be to assume that the response to quality is proportional to quantity applied, in which case these differences should be, in the present example, in the ratio 1:2:3. To meet this, we now propose an alternative model:—

Qualities	Quantities			
	1	2	3	
A	$m + b_1$	$m + 2b_1$	$m + 3b_1$	(Model 3)
B	$m + b_2$	$m + 2b_2$	$m + 3b_2$	
Differences	Δb	$2\Delta b$	$3\Delta b$	

Fitting the constants m, b_1 , and b_2 to a set of data would be equivalent to fitting two linear regression lines to the two sets of data supplied by the two qualities, but with the restriction that the vertical differences between the lines at $x = 1, 2, 3$ must be in the ratio 1:2:3, or, in other words, that the lines must have a common point at $x = 0$.

The observational equations under the hypothesis of proportional response, with all other deviations considered random, are therefore:—

$$\begin{matrix} m & b_1 & b_2 \end{matrix}$$
$$\begin{bmatrix} 1 & 1 & \cdot \\ 1 & 2 & \cdot \\ 1 & 3 & \cdot \\ 1 & \cdot & 1 \\ 1 & \cdot & 2 \\ 1 & \cdot & 3 \end{bmatrix} = \begin{bmatrix} y_{11} \\ y_{12} \\ y_{13} \\ y_{21} \\ y_{22} \\ y_{23} \end{bmatrix}$$

The normal equations are:—

$$\begin{bmatrix} 6 & 6 & 6 \\ 6 & 14 & \cdot \\ 6 & \cdot & 14 \end{bmatrix} = \begin{bmatrix} Y \\ y_{11} + 2y_{12} + 3y_{13} \\ y_{21} + 2y_{22} + 3y_{23} \end{bmatrix}$$

Solving these, we have

$$14(b_1 + b_2) = Y_{01} + 2Y_{02} + 3Y_{03} - 12m,$$

whence $$42m - 36m + 3(Y_{01} + 2Y_{02} + 3Y_{03}) = 7Y$$

$$m = \frac{7}{6} Y - \frac{1}{2}(Y_{01} + 2Y_{02} + 3Y_{03})$$

$$b_1 = \frac{1}{14}\{y_{11} + 2y_{12} + 3y_{13} + 3(Y_{01} + 2Y_{02} + 3Y_{03}) - 7Y\}$$

$$b_2 = \frac{1}{14}\{y_{21} + 2y_{22} + 3y_{23} + 3(Y_{01} + 2Y_{02} + 3Y_{03}) - 7Y\}$$

The quality difference, $\Delta b = b_1 - b_2$, is therefore estimated as

$$\frac{1}{14}(\delta y_1 + 2\delta y_2 + 3\delta y_3).$$

The fitting of these constants (or these two linear regression lines) absorbs 2 degrees of freedom, if we exclude that for m, which is merely equivalent to a change of origin similar to that for the correction factor in an ordinary sum of squares. There remains a third degree of freedom available from the previous model, which represents differences between the lines fitted on the basis of the present model and the best fitting lines provided by Model 2; in other words it represents deviations from

the ratio 1:2:3 of quality differences for the different quantity levels. This is the so-called "quality × quantity interaction".

An additional constant may be introduced to represent such deviations. Taking the first quality only, we may propose the amended model

$$m + b_1 + k_1 d_1 \qquad m + 2b_1 + k_2 d_1 \qquad m + 3b_1 + k_3 d_1$$

where k_1 , k_2 , k_3 are fixed for a given interaction table, and are determined as follows:—

(1) There must be no component of d_1 in the expression used to estimate quality differences, viz. $y_{11} + 2y_{12} + 3y_{13}$.

Hence $k_1 + 2k_2 + 3k_3 = 0$.

(2) Since we are still fitting linear regression lines, we must have

$$k_2 - k_1 = k_3 - k_2$$

These equations yield the integral solution

$$k_1 = 4, \qquad k_2 = 1, \qquad k_3 = -2,$$

and the model becomes (with d_1 and d_2 constrained to sum to zero):

Qualities	Quantities			
	1	2	3	
A	$m + b_1 + 4d_1$	$m + 2b_1 + d_1$	$m + 3b_1 - 2d_1$	(Model 4)
B	$m + b_2 + 4d_2$	$m + 2b_2 + d_2$	$m + 3b_2 - 2d_2$	

For this model the normal equations are:—

$$\begin{array}{ccccc} m & b_1 & b_2 & d_1 & d_2 \end{array}$$

$$\begin{bmatrix} 6 & 6 & 6 & \cdot & \cdot \\ 6 & 14 & \cdot & \cdot & \cdot \\ 6 & \cdot & 14 & \cdot & \cdot \\ 3 & \cdot & \cdot & 21 & \cdot \\ 3 & \cdot & \cdot & \cdot & 21 \end{bmatrix} = \begin{bmatrix} Y \\ y_{11} + 2y_{12} + 3y_{13} \\ y_{21} + 2y_{22} + 3y_{23} \\ 4y_{11} + y_{12} - 2y_{13} \\ 4y_{21} + y_{22} - 2y_{23} \end{bmatrix}$$

The values for m, b_1, and b_2 are unaltered. The remainder of the solution is

$$d_1 = \frac{1}{21}(4y_{11} + y_{12} - 2y_{13} - 3m)$$

$$d_2 = \frac{1}{21}(4y_{21} + y_{22} - 2y_{20} - 3m).$$

The sum of d_1 and d_2 is zero, as required.

The linear functions having detached coefficients (4,1, − 2), orthogonal to (1,2,3), correspond to the subdivision proposed by Fisher (4).

NUMERICAL ILLUSTRATIONS

Example (1).

The following table shows the yields of a single cut from an experiment on natural pasture. Ammonium sulphate (S) was applied at 300, 600, and 900 lbs. per acre and Ammonium nitrate (N) at equivalent rates.

Qualities	Quantities			Totals	(1) + 2(2) + 3(3)	4(1) + (2) − 2(3)
	1	2	3			
S	197.61	218.04	223.97	639.62	1,305.60	560.54
N	186.20	215.63	217.86	619.69	1,271.04	524.71
Totals	383.81	433.67	441.83	1,259.31	2,576.64	1,085.25

(a) Using Model 3:—

$$6m = 1{,}259.31 \times 7 - 2{,}576.64 \times 3 = 1{,}085.25,$$

(= total of last column)

whence $m = 180.88$

$$b_1 = \frac{1}{14}(1{,}305.60 - 1{,}085.25) = 15.74$$

$$b_2 = \frac{1}{14}(1{,}271.04 - 1{,}085.25) = 13.27.$$

Hence the fitted values are:—

Qualities	Quantities		
	1	2	3
S	196.62	212.36	228.10
N	194.15	207.42	220.69
Differences	2.47	4.94	7.41

The differences are in the ratio 1:2:3 as required.

(b) Using Model 4:—

We find

$$d_1 = \frac{1}{21}(560.54 - 542.62) = 0.85$$

$$d_2 = \frac{1}{21}(524.71 - 542.62) = -0.85,$$

m, b_1 , and b_2 being as before. The fitted values are then:—

Qualities	Quantities		
	1	2	3
S	200.02	213.21	226.40
N	190.75	206.57	222.39

(c) Using Model 2:—

We find

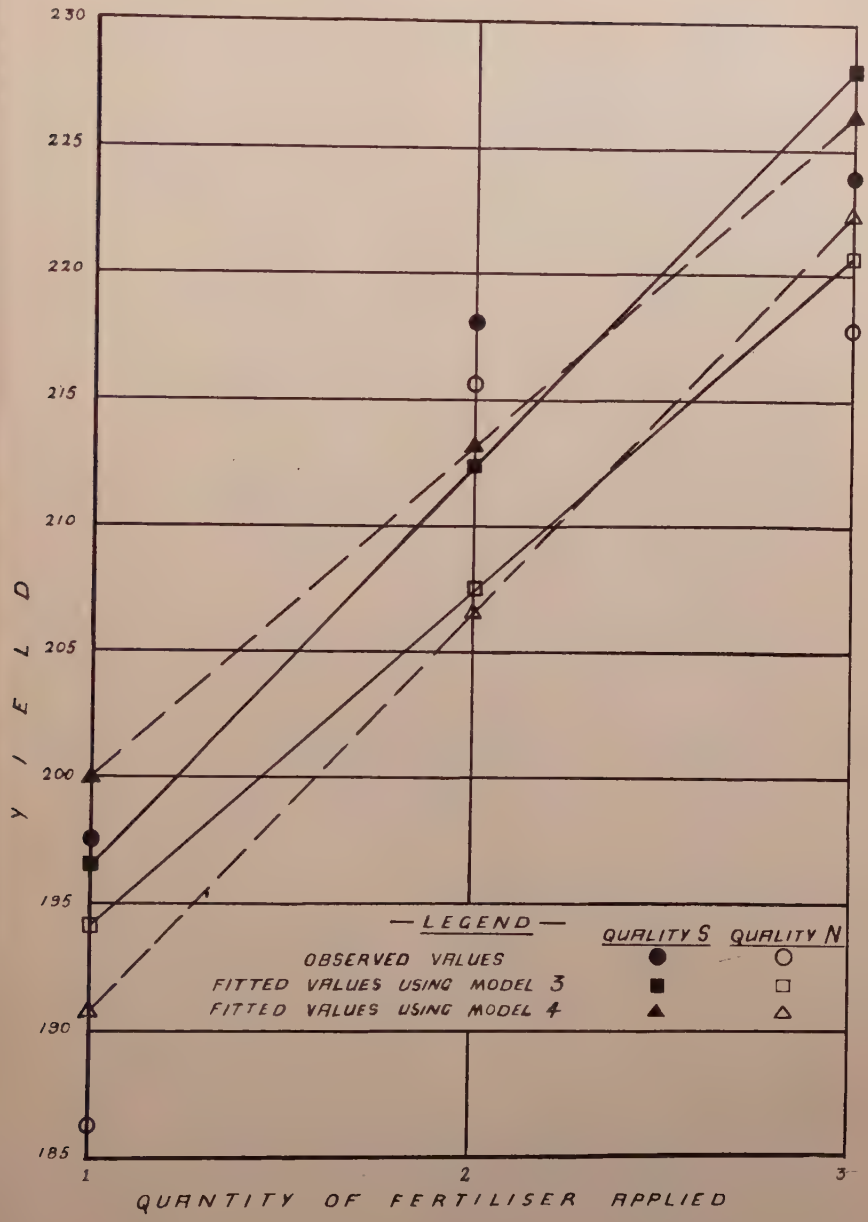
$$m = \frac{1{,}259.31}{6} = 209.88$$

$$q = \tfrac{1}{6}(619.69 - 639.62) = -3.32$$

$$b = 14.50$$

$$\delta b = 1.32.$$

It may be confirmed that apart from rounding errors the fitted values obtained from this model are identical with those obtained by using Model 4.



These fitted values have been plotted in the diagram. The effect of the quality difference is revealed in the comparison of the two continuous lines, firstly by their width apart and secondly by the angle

between them. These two criteria are obviously not independent, however, since an increase of the distance between the lines at $x = 1$ must increase the angle between the lines if the ratio 1:2:3 of vertical distances between the lines at $x = 1, 2, 3$ is maintained. This is clear from the fact that in testing the significance of the quality difference, we are testing the difference between b_1 and b_2 of Model 3, and this difference not only measures the difference in slopes of the two regression lines, but also the distance between them.

From the diagram it appears that the broken lines are somewhat dissimilar to the continuous lines, providing evidence that the hypothesis of proportional response to quality is not obeyed as closely as it might be.

Hence it may be stated:—

1. The test of significance of the quality difference tests the significance of the difference in position and slope of 2 linear regression lines fitted to the data for the 2 qualities on the assumption of proportional response to quality.
2. The test of significance of the quality × quantity interaction tests the validity of the assumption that qualitative responses are proportional to quantity applied. Should this interaction prove significant, the assumption of proportional response can no longer be considered valid and the previous test and estimates of qualitative effects will become meaningless. On the other hand, irrespective of the significance or otherwise of the test for effect of quality, the significance of the interaction would be clear evidence of some kind of qualitative effect.

In the numerical example the yields were totals of 12 plots, so that the sums of squares for the various effects are

$$\text{S. S. Quality} = \frac{(1{,}305.60 - 1{,}271.04)^2}{2 \times 14 \times 12} = 3.5547$$

$$\text{S. S. Quality} \times \text{quantity} = \frac{(560.54 - 524.71)^2}{2 \times 21 \times 12} = 2.5472,$$

each with one degree of freedom. (In practice a row of differences $(S - N)$ would be added to the interaction table).

These two sums of squares must add to the same as the sums of squares for quality and differences of linear effects appropriate to Model 2, namely to

$$\frac{1}{72}(639.62 - 619.69)^2 + \frac{1}{48}(197.61 - 186.20 - 223.97 + 217.86)^2$$

$$= 5.5167 + 0.5852,$$

checking the computations. It would thus appear that in this example the ordinary additive hypothesis is nearer the truth, since it gives the higher sum of squares for quality, but in actual fact all of the above mean squares were less than the error mean square of the experiment. The illustration is therefore not a good one in respect of demonstrating experimental agreement with the proportional hypothesis. It was chosen so that the points and lines of the diagram would be distinct, and also because the ratio of quantities applied corresponds to that of the theoretical discussion, which in turn discusses the particular case dealt with by Fisher. In many examples the points and lines are so close to one another that a clear diagram would be impossible. In any case it does sometimes happen that qualitative differences observed at the lowest level keep more or less constant at the other levels, especially when there is comparatively little response to the additional applications. (See Example 3).

Example 2. Yields of maize (in lb.) are given for superphosphate and rockphosphate each applied at the rates 100, 300, and 500 lb. per acre.

Qualities	Quantities			Totals	(1) + 3(2) + 5(3)	13(1) + 4(2) − 5(3)
	1	2	3			
S	84.73	85.05	89.09	258.87	785.33	996.24
R	83.45	82.00	82.93	248.38	744.10	998.20
$S + R$	168.18	167.05	172.02	507.25	1,529.43	1,994.44
$S - R$	1.28	3.05	6.16	10.49	41.23	−1.96

In this example Model 4 needs to be amended to suit the 1:3:5 ratio of quantities. Thus, here $k_1 = 13$, $k_2 = 4$, $k_3 = -5$, and the normal equations are:

$$\begin{array}{ccccc} m & b_1 & b_2 & d_1 & d_2 \\ \begin{bmatrix} 6 & 9 & 9 & \cdot & \cdot \\ 9 & 35 & \cdot & \cdot & \cdot \\ 9 & \cdot & 35 & \cdot & \cdot \\ 12 & \cdot & \cdot & 210 & \cdot \\ 12 & \cdot & \cdot & \cdot & 210 \end{bmatrix} & = & \begin{bmatrix} 507.25 \\ 785.33 \\ 744.10 \\ 996.24 \\ 998.20 \end{bmatrix} \end{array}$$

The solution is $m = 83.102$, $b_1 = 1.069$, $b_2 = -0.109$, $d_1 = -0.005$ $d_2 = 0.005$. The interaction components are very small, and the fitted values

Qualities	Quantities		
	1	2	3
S	84.11	86.12	88.68
R	83.05	82.96	82.33

are close to the observed yields.

Sums of squares are computed as follows:—

$$\text{S.S. Quality} = \frac{(41.23)^2}{2 \times 35 \times \frac{1}{4}} = 97.14$$

$$\text{S.S. Quality} \times \text{quantity} = \frac{(1.96)^2}{2 \times 210 \times \frac{1}{4}} = 0.04,$$

since each yield in the table is a mean of 4 plots. The error mean square of the experiment was 19.38 with 56 D.F., and so the quality difference is significant at the 1% level. The average response to 100 lb. superphosphate was 1.069 lb. per plot, whereas 100 lb. of rock phosphate depressed the yield by 0.109 lb. on the average. The mean response to 100 lb. of phosphate (average of both qualities) is 0.480 lb. per plot, which corresponds to the average response given by b in Model 2—in this example $\frac{1}{8}(172.02 - 168.18) = 0.480$. This is a consequence of the fact that the common regression line of Model 2 is an average for both pairs of regression lines (distinguished by continuous and broken lines in the diagram).

Standard errors of these estimates may be found by expressing b_1 , b_2 , etc. as linear functions of the yields in the interaction table. Thus

$$35b_1 = y_{11} + 3y_{12} + 5y_{13} - \frac{9}{24}(13Y_{01} + 4Y_{02} - 5Y_{03}),$$

i.e.

$$b_1 = \frac{1}{8 \times 35}(-31y_{11} - 39y_{21} + 12y_{12} - 12y_{22} + 55y_{13} + 15y_{23}),$$

so that

$$\text{Var. } (b_1) = \frac{1}{8^2 \times 35^2}(31^2 + 39^2 + \cdots + 15^2)\sigma^2/4,$$

where σ^2 is the variance of a single plot,

$$= 0.01920\sigma^2.$$

Hence S.E. of b_1 or b_2 $= \sqrt{0.01920 \times 19.38} = 0.610$.
Variance of average response, $\frac{1}{2}(b_1 + b_2)$,

$$= \frac{1}{64} \times 2 \times \frac{\sigma^2}{4}; \qquad \text{S.E.} = 0.389.$$

Variance of difference of average responses, $b_1 - b_2$,

$$= \frac{1}{35} \times 2 \times \frac{\sigma^2}{4}; \qquad \text{S.E.} = 0.526.$$

QUADRATIC AND HIGHER EFFECTS

So far the problem has been considered on the basis of purely linear responses to quantity, but in actual fact the regressions may be non-linear. In the general case, if the overall regression (i.e. for all qualities combined) is significantly non-linear, then there will be curvature of the regression lines for at least some of the qualities. These curvatures may be similar or different.

The fitting of constants to represent curvature effects may be simply done by the use of orthogonal polynomials. Since the degrees of freedom representing differences of curvature will then be orthogonal to those representing linear effects and differences of linear effects, they will also be orthogonal to those representing quality and interaction of quality and quantity as defined above.

In the numerical example we may therefore propose constants to represent quadratic effects, whereupon the model will become:—

Quality	Quantity		
	1	2	3
A	$m + b_1 + 4d + c_1$	$m + 2b_1 + d - 2c_1$	$m + 3b_1 + 2d + c_1$
B	$m + b_2 - 4d + c_2$	$m + 2b_2 - d - 2c_2$	$m + 3b_2 + 2d + c_2$

Here a single constant d has replaced the two constants d_1 and d_2 ($d_1 + d_2 = 0$). In the analysis the 2 degrees of freedom for c_1 and c_2

will be presented as one degree of freedom representing an overall quadratic effect (the mean of c_1 and c_2), and a second degree of freedom representing the difference between c_1 and c_2 .

Should neither of these degrees of freedom prove to be significant, the analysis based on the assumption of purely linear regressions will be valid, but should either test of significance prove significant, this result will supersede previous findings based on linearity alone. In the case of a significant difference between the quadratic effects there will also be evidence of differential qualitative effects. The same applies to cubic and higher effects in the general case.

If we estimate c_1 and c_2 in the numerical example by applying the orthogonal polynomial values (1, − 2, 1) to the yields for each quality, it will be found that the fitted values correspond exactly to the observed values, since a quadratic curve can be made to pass through any set of 3 points. The sums of squares for average and difference of quadratic effects in the two examples given earlier are:—

Example 1:

$$\text{S.S. Average quadratic effect} = \frac{(383.81 - 2 \times 433.67 + 441.83)^2}{6 \times 24}$$

$$= 12.0756$$

S.S Difference of quadratic effects (or Quadratic effect × quality)

$$= \frac{(197.61 - 2 \times 218.04 + 223.97 - 186.20 + 2 \times 215.63 - 217.86)^2}{144}$$

$$= 1.1201$$

Example 2:

$$\text{S.S. Average quadratic effect} = \frac{(168.18 - 2 \times 167.05 + 172.02)^2}{6 \times \frac{1}{2}}$$

$$= 12.40$$

$$\text{S.S. Difference of quadratic effects} = \frac{(1.28 - 2 \times 3.05 + 6.16)^2}{3}$$

$$= 0.60$$

Hence in neither example is there any significant evidence of curvature, though the reason for the relatively high sum of squares for average curvature in Example 1 is apparent from the diagram.

DUMMY TREATMENTS

If a zero quantity is included, it is usual to include one such plot for each quality in the experiment. This enables the overall linear, quadratic, etc. effects to be computed without difficulty by the use of ordinary orthogonal polynomial values, since the observations at each level are of equal weight. It will also probably be necessary from the point of view of arranging a suitable confounded design. In this case, provided that none of the totals in the interaction table is subject to block effects, differences between the treatment totals for the zero application will be due to experimental error. A sum of squares due to "dummies" is therefore separated out for inclusion in the error sum of squares.

Occasionally in experimental work more than one set of control or zero-application treatments may have to be included. It may not be inappropriate therefore to consider the subdivision of sums of squares for the interaction table in a general case. Let P and Q be 2 factors occurring at p and q levels, respectively, and let the interaction table of yields be as follows:—

Level of Factor P	Level of Factor Q 1	2	·····	q	Totals
1	y_{11}	y_{12}	·····	y_{1q}	Y_{10}
2	y_{21}	y_{22}	·····	y_{2q}	Y_{20}
·	·	·	·	·	·
·	·	·	·	·	·
·	·	·	·	·	·
·	·	·	·	·	·
·	·	·	·	·	·
·	·	·	·	·	·
p	y_{p1}	y_{p2}	·····	y_{pq}	Y_{p0}
Totals	Y_{01}	Y_{02}	·····	Y_{0q}	Y

The y_{ij} are for simplicity assumed to be from a single replication. P and Q are factors of such type that, if a zero application of Q occurs, then automatically it is associated with a zero application of P.

Let us suppose that the first r levels of factor Q, but none of factor P, are zero or control levels. Then we may compute the following sums of squares:—

$$\text{S.S. All treatment combinations} = \sum_i \sum_j y_{ij}^2 - \frac{1}{pq} Y^2 = A, \quad \text{with } pq - 1 \text{ D.F.}$$

$$\text{S.S. Factor } Q = \frac{1}{p} \sum_i Y_{0i}^2 - \frac{1}{pq} Y^2 = B, \quad \text{with } q - 1 \text{ D.F.}$$

B may be subdivided into

$$\text{S.S. Controls v. Remainder} = \frac{\left[(q-r) \sum_{j=1}^{r} Y_{0j} - r \sum_{j=r+1}^{q} Y_{0j}\right]^2}{p[r(q-r)^2 + (q-r)r^2]} = C, \quad \text{with 1 D.F.}$$

S.S. Controls among themselves

$$= \frac{1}{p} \sum_{j=1}^{r} Y_{0j}^2 - \frac{1}{pr} \left(\sum_{j=1}^{r} Y_{0j} \right)^2 = D, \quad \text{with } r - 1 \text{ D.F.}$$

S.S. Remainder among themselves

$$= \frac{1}{p} \sum_{j=r+1}^{q} Y_{0j}^2 - \frac{1}{p(q-r)} \left(\sum_{j=r+1}^{q} Y_{0j} \right)^2 = E, \quad \text{with } q - r - 1 \text{ D.F.}$$

Thus $B = C + D + E$.

S.S. Factor P (ignoring the fact that some levels of Q are zero levels)

$$= \frac{1}{q} \sum_i Y_{i0}^2 - \frac{1}{pq} Y^2 = F, \quad \text{with } p - 1 \text{ D.F.}$$

The correct S.S. for factor P (allowing for dummies)

$$= \frac{1}{q-r} \left(\sum_i \sum_{j=r+1}^{q} y_{ij} \right)^2 - \frac{1}{p(q-r)} \left(\sum_{j=r+1}^{q} Y_{0j} \right)^2 = G, \quad \text{with } p - 1 \text{ D.F.}$$

S.S. for Interaction PQ (ignoring dummies)

$$= H = A - B - F, \quad \text{with } (p-1)(q-1) \text{ D.F.}$$

The correct S.S. for Interaction PQ (allowing for dummies)

$$= \sum_i \sum_{j=r+1}^{q} y_{ij}^2 - \frac{1}{p(q-r)} \left(\sum_{j=r+1}^{q} Y_{0j} \right)^2 - E - G$$

$$= I, \quad \text{with } (p-1)(q-1) \text{ D.F.}$$

i.e. it is computed in the ordinary way from a truncated interaction table in which the control treatments are excluded.

In addition there is a sum of squares representing differences of control plots for the various levels of P

$$= \sum_{i} \sum_{j=1}^{r} y_{ij}^2 - \frac{1}{p} \sum_{j=1}^{r} Y_{0j}^2 = J, \qquad \text{with } (p-1)(r-1) \text{ D.F.}$$

The sums of squares J and D represent dummy comparisons and must be included in error.

It can readily be seen that not only is A equal to $B + F + H$, representing the ordinary subdivision for an interaction table, but also to $B + G + I + J$.

Consider now the very common case where Q is a quantitative factor with lowest level zero, and P is a factor representing different qualities of Q. With only a single control level, D does not exist. A possible method of subdivision of the sum of squares of such an interaction table would be into $B + G + I + J$, but with B preferably subdivided into polynomial effects (linear, quadratic, etc.) rather than into $C + E$, and with $G + I$ alternatively subdivided into sums of squares for Quality, Quality × quantity, Quality × quadratic effect, etc. as indicated earlier. With minor differences this is actually the type of analysis suggested by Bliss (1) for slope-ratio assays, except that the sum of squares for Quality × quantity (labelled "non-convergence at zero dose") is there obtained by a subtraction method. However, such a subdivision is in general not completely satisfactory, being practically equivalent to treating the zero quantitative level as a qualitative control, except for the fact that the average effect of quantities is estimated over a wider range by treating the control as a zero level. By this method we would not be considering the zero level when estimating quadratic and higher degree regression effects of the individual qualities. Occasionally (for example, when there is a big response to the first level of application followed by moderate responses to subsequent levels), this may be an advantage, since a polynomial curve might be unsuitable as a representation of the data over the whole range of applications, but adequate over the part of the range excluding zero.

The above consideration seems to lie behind the change of nomenclature introduced by Cochran and Cox whereby the sums of squares in a 3 × 3 table with a zero level, which Fisher calls "Quality" and "Quality × quantity", are renamed "Differences in linear response" and "Differences in curvature". The extension to more than three levels is thus suggested and will be illustrated by example. In both the

experiments used earlier there were actually zero levels which were previously suppressed in order to provide the type of example required.

Example 1: The yields of the two zero levels were 123.26 and 109.88.

$$\text{S.S. Dummies} = \frac{(123.26 - 109.88)^2}{24} = 7.4593$$

S.S. Linear effect of nitrogen (N')

$$= \frac{(-3 \times 233.14 - 383.81 + 433.67 + 3 \times 441.83)^2}{20 \times 24}$$

$$= 951.8362$$

S.S. Quadratic effect of nitrogen (N'')

$$= \frac{(233.14 - 383.81 - 433.67 + 441.83)^2}{4 \times 24}$$

$$= 211.5531$$

S.S. Cubic effect of nitrogen (N''')

$$= \frac{(-233.14 + 3 \times 383.81 - 3 \times 433.67 + 441.83)^2}{20 \times 24}$$

$$= 7.2792$$

The regression effects above are obtained as usual by applying the appropriate orthogonal polynomial values to the quantity totals.

It is now proposed to subdivide the sum of squares $G + I$ with 3 D.F. by applying to the differences $S - N$ at the three non-zero levels the set of coefficients

$$\begin{matrix} 1 & 2 & 3 \\ -1 & -1 & 1 \\ 3 & -3 & 1 \end{matrix} \qquad (1)$$

instead of the set

$$\begin{matrix} 1 & 2 & 3 \\ 4 & 1 & -2 \\ 1 & -2 & 1 \end{matrix} \qquad (2)$$

It will be noticed that, whereas set (2) is completely orthogonal, set (1) is not, because the second and third rows consist of the quadratic and cubic orthogonal polynomials for 4 equally spaced levels less the first coefficient, which cancels owing to the common zero level. Thus the sum of squares for "Differences in linear effect" (or Quality) is orthogonal to those for "Differences in quadratic effect" and "Differ-

ences in cubic effect", but the last two are not orthogonal to one another. It would be possible either to combine these into a joint sum of squares for interaction with 2 D.F., obtained by subtraction, or to compute "Differences in quadratic effect ignoring differences in cubic (and, in general, higher) effects", leaving a residual interaction sum of squares. The latter course is probably preferable, since effects higher than quadratic, and consequently differences of these, are likely to be negligible. There is, however, no independent check on the computations in either of these cases.

Hence we compute as follows:—

S.S. Differences in linear effect = 3.5547 (as before)
S.S. Differences in quadratic effect ignoring differences in cubic effect

$$= \frac{(-11.41 - 2.41 + 6.11)^2}{3 \times 24} = 0.8256$$

Residue (differences in cubic effect eliminating differences in quadratic effect) = 2.8418.

Only N' and N'' are significant in this experiment, and the significance of N'' shows that the average response to 300 lb. of nitrogen was not consistent over the range studied. The sum of squares $G + I$ was, in fact, not as great as the error mean square and so in practice no subdivision would be necessary.

Example 2: The quantities are in the ratio 0:1:3:5, and only 4 plots with a mean yield of 78.20 were available at the zero level; there is thus no sum of squares for "Dummies". The computations are simplified by using the appropriate weighted orthogonal polynomial coefficients. These are (to be applied to marginal totals):—

Linear	−18	−11	3	17	(Divisor 1,162)
Quadratic	608	−108	−544	348	(Divisor 1,227,072)
Cubic	−30,720	28,800	−19,200	5,760	(Divisor 3,406,233,600)

The divisors refer to a single entry in the interaction table, and must actually be divided by 4, since each entry is the mean of 4 plots.

S.S. Linear effect of phosphate (P')

$$= \frac{(-18 \times 78.20 - 11 \times 168.18 + 3 \times 167.05 + 17 \times 172.02)^2}{1{,}162 \times \frac{1}{4}}$$

$$= \frac{(167.91)^2}{290.5} = 97.05.$$

Similarly, S.S. $P'' = 8.66$.

S.S. $P''' = 59.32$.

The sum, $P' + P'' + P'''$, checks to the S.S. for Quantities, 165.04.

S.S. Differences in linear effect = 97.14 (as before).

S.S. Differences in quadratic effect ignoring differences in cubic effect

$$= \frac{(-108 \times 1.28 - 544 \times 3.05 + 348 \times 6.16)^2}{(108^2 + 544^2 + 348^2) \times 2 \times \frac{1}{4}}$$

$$= 0.56.$$

Residue $= 0.08$.

In this experiment it is clear that the two qualities of phosphate have given significantly different average responses to 100 lbs. applied. There is no evidence of any variation in these responses; for P''', although sizeable, is not significant.

Although the sum of squares for differences of linear effects (or quality) is the same as before, the estimates of the two average responses, b_1 and b_2 , are different in consideration of the additional information provided by the zero level. Amending the previous notation for the yields of the interaction table by the addition of y_{00} for the yield at zero level (which will now be included in the total, Y), we find the normal equations for m, b_1 , and b_2 to be

$$\begin{matrix} m & b_1 & b_2 \end{matrix}$$

$$\begin{bmatrix} 7 & 9 & 9 \\ 9 & 35 & \cdot \\ 9 & \cdot & 35 \end{bmatrix} = \begin{bmatrix} 585.45 \\ 785.33 \\ 744.10 \end{bmatrix},$$

whence $m = 81.035$, $b_1 = 1.600$, $b_2 = 0.422$. The difference $b_1 - b_2$ is, of course, the same as before and has the same S.E. To find the S.E. of b_1 or b_2 we have

$$b_1 = \frac{1}{35}\left\{y_{11} + 3y_{12} + 5y_{13} + \frac{81}{83}(Y_{01} + 3Y_{02} + 5Y_{03}) - \frac{315}{83}Y\right\}.$$

$$= \frac{1}{35 \times 83}(-315y_{00} - 151y_{11} - 234y_{21} + 177y_{12} - 72y_{22}$$

$$+ 505y_{13} + 90y_{23}).$$

Hence Var. $(b_1) = 0.01411\sigma^2$,

$$\text{S.E.} = 0.523.$$

Variance of average response, $\frac{1}{2}(1.600 + 0.422) = 1.011$, which is the same thing as (7/1162) (167.91),

$$= \frac{7^2}{1162} \cdot \frac{\sigma^2}{4},$$

$$\text{S.E.} = 0.452.$$

Example 3. The following interaction table is from an experiment on wattle with 5 levels of phosphatic fertilizer (nil, 200, 400, 600, 800 lbs. per acre), the plots of which were split for the comparison of 2 different types of phosphate. The figures represent mean heights per sub-plot at one year totalled over 9 sub-plots.

Qualities	Quantities					Totals
	0	1	2	3	4	
A	67.1	77.9	77.7	77.5	78.5	378.7
B	67.9	83.1	83.0	84.2	84.5	402.7
Totals	135.0	161.0	160.7	161.7	163.0	781.4
Differences	0.8	5.2	5.3	6.7	6.0	24.0

A subdivision of the sum of squares for quantities by means of orthogonal polynomials resulted in the following analysis of variance:—

Source of variation	D.F.	Sum of squares	M.S.	F.
Linear	1	17.861	17.861	30.8**
Quadratic	1	9.181	9.181	15.9**
Cubic and quartic	2	4.580	2.290	4.0*
Total	4	31.622		
Error (a)	14		0.5791	

The significance of the residual mean square shows that the routine procedure of fitting a second degree curve is inadequate. Inspection of the data reveals very little response to phosphate after the first 200 lb., and so we subdivide alternatively as follows:—

Source of variation	D.F.	S.S.	M.S.
Phosphate v. no phosphate	1	31.447	
Between phosphate levels	3	0.175	
Total	4	31.622	
Error (a)	14		0.5791

It is evident that the variation between the non-zero quantities is small compared with experimental error. In this example, therefore, by way of an exception, it seems preferable to keep the zero level apart from the others, subdividing the 2 × 4 table excluding the zero level according to the method discussed earlier in the paper. This results as follows:—

Source of variation	D.F.	S.S.	M.S.	F.
Types of phosphate	1	6.645	6.645	32.9**
Quality × quantity	1	0.871	0.871	4.3*
Quality × quadratic effect	1	0.009		
Quality × cubic effect	1	0.032		
Total	4	7.557		
Error (b)	25		0.2022	

The significant quality × quantity interaction shows that the proportional hypothesis is not obeyed here, and an inspection of the quality differences shows that the simple additive hypothesis will probably be closer to the mark, as might reasonably be expected in view of the lack of response to additional applications of phosphate.

The new subdivision on this hypothesis is:—

Source of variation	D.F.	S.S.	M.S.	F.
Types of phosphate	1	7.476	7.476	37.0**
Quality × quantity	3	0.081		
Total	4	7.557		
Error (b)	25		0.2022	

Hence in this experiment an adequate measure of the qualitative difference is provided by an unweighted mean of the quality differences at the different quantitative levels.

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SUMMARY

A method proposed by Fisher of estimating the effects of quality and the interaction of quality and quantity in experiments containing a number of qualities of a fertiliser at a number of quantitative levels is discussed, and the interpretation of these estimates from the experimental point of view is considered.

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STRAWBERRY UNIFORMITY YIELD TRIALS[1]

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Realistic and comprehensive mathematical models for the analyses of field trial data can be based only on an extensive and intensive study of uniformity trials. The collection of uniformity trial data is a time and money consuming project. It cannot be assumed that the models found adequate for one crop in one year at one place can be extrapolated with any degree of confidence to other quite different crops in very different environments. This is a well recognized problem and several workers (1), (2), (3), (4), (5), (6), (7), (8), and (9) have published recent results.

The purpose of the present paper is to present two uniformity yield trials for strawberries. The variety grown was the same in the two cases but the environments were somewhat different for the different trials. Some calculations are made and presented including random sampling results in order to emphasize differences between the trials and point to possible arrangements of field trials that will lead to improved clarity of interpretation.

DATA

The data consist of individual plant yields in grams for two uniformity trials. One consisted of 200 plants of the Tahoe variety grown in two double-row beds at Davis in 1946. The beds of two rows, which were one foot apart, were spaced 42 inches apart with 10 inches between plants in the rows. The rows consisted of 50 plants. These data are presented in Table 1.

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[1]This paper was read at a joint meeting of the Institute of Mathematical Statistics and the Western North American Region of The Biometric Society held June 15–16, 1951, at the Rand Corporation, Santa Monica, California, and an abstract appeared in *Biometrics* 7(3):300.

TABLE 1. TWO HUNDRED INDIVIDUAL PLANT YIELDS IN GRAMS
DAVIS, 1946

Double Bed No. 1		Double Bed No. 2	
176	145	141	139
235	176	207	138
217	96	155	167
234	205	211	218
239	207	261	158
216	212	197	173
318	138	295	152
190	136	161	191
181	145	228	296
225	166	300	160
266	317	274	163
297	143	249	245
226	228	211	268
283	245	235	252
219	167	236	159
267	157	140	275
265	144	125	185
216	190	67	203
130	215	273	132
148	191	184	234
174	212	213	199
246	207	278	276
198	228	181	242
194	213	263	159
260	174	228	193
245	87	142	241
207	154	269	211
203	212	287	243
260	155	290	176
204	160	226	272
136	87	228	270
243	129	213	352
325	251	165	256
106	295	208	220
222	185	254	277

TABLE 1—*Continued*

Double Bed No. 1		Double Bed No. 2	
228	235	161	295
124	139	242	239
288	97	280	273
240	194	237	159
181	175	185	244
231	95	193	250
193	105	160	261
144	163	204	252
208	271	228	185
191	207	226	182
166	117	128	195
216	152	298	280
282	148	293	196
232	151	267	199
284	150	278	183

The second uniformity trial consisted of 500 plants of the Tahoe variety grown in single beds at Davis in 1948. The beds were 30 inches apart with 10 inches between plants. The rows were 50 plants long. The irrigation water was somewhat saline which may have increased the variability of plant yields. These data are presented in table 2.

The mean yield in grams for the two trials were 208 and 173 respectively. The ratio of the standard deviation to the mean was 0.26 for the 200-plant trial and 0.39 for the 500-plant trial.

RANDOM SAMPLING RESULTS

Various sizes and arrangements of plots were imposed on both of the uniformity trials and "varieties" assigned to the elementary plots at random. Conventional F-values were calculated and the results are given in tables 3 and 4. F-values using both residual error (internal variance) and replication x variety interaction are given simply to call attention to differences between the two trials and the various arrangement of plots.

It should be noted that in general with q varieties and p replications $(q!)^p$ arrangements are possible. Since in the present case if we have a particular set up we can rearrange the "variety" designations in $q!$ ways and get the same F-value. It follows that there are $(q!)^{p-1}$ distinct

TABLE 2. FIVE HUNDRED INDIVIDUAL PLANT YIELDS IN GRAMS, DAVIS, 1948
SINGLE BEDS 30 INCHES APART

129	203	126	136	141	81	88	144	225	80
72	95	71	249	109	47	240	121	167	71
124	130	106	88	197	154	160	142	233	20
95	94	87	57	97	156	201	284	230	124
100	126	73	94	150	163	109	143	170	175
63	71	153	93	173	181	324	197	203	171
76	136	138	39	74	229	192	275	193	115
153	46	92	55	117	106	221	207	245	143
88	149	122	96	186	160	311	206	285	175
58	116	149	123	136	226	373	319	286	211
72	114	142	67	94	253	201	249	154	39
178	215	197	106	160	229	192	188	206	177
194	175	132	147	137	225	193	157	151	260
193	220	131	123	182	208	293	326	158	256
205	180	117	145	240	402	339	127	232	186
212	234	127	114	258	241	168	244	220	194
206	100	183	216	234	212	210	197	188	137
171	169	106	250	190	161	204	132	279	201
119	228	231	149	163	230	299	220	130	159
155	269	110	92	316	192	299	214	237	94
155	127	223	102	251	126	155	216	150	170
251	122	320	338	317	107	270	116	294	172
275	117	209	136	191	153	179	132	293	90
278	91	194	175	122	265	208	105	199	265
324	128	203	210	211	145	233	196	230	132
190	221	240	129	82	253	144	148	76	268
134	243	251	171	208	97	115	262	231	193
189	295	76	108	168	248	304	170	155	112
141	97	108	169	156	189	63	239	211	43
180	286	148	142	156	112	131	166	79	80
175	265	201	193	220	114	212	97	118	59
238	317	204	184	224	216	87	193	194	87
272	185	208	128	178	266	135	209	124	74
206	194	272	123	197	182	97	111	128	132
78	293	120	173	151	135	261	57	59	224

TABLE 2—*Continued*

58	136	220	169	135	179	259	199	201	180
146	344	120	222	201	144	155	187	215	239
171	126	148	289	242	149	133	243	289	102
321	225	96	230	128	72	375	171	299	247
275	333	166	219	113	187	217	122	191	105
108	249	128	139	223	56	78	212	119	90
53	128	234	173	163	164	203	55	183	195
128	225	190	107	189	246	194	178	149	232
88	185	317	116	246	211	79	180	213	44
206	215	137	222	251	255	285	231	172	135
104	171	134	83	172	185	194	172	315	60
112	104	289	172	119	191	243	196	124	96
52	151	306	209	207	113	189	209	215	21
68	117	198	202	136	118	241	172	133	162
82	144	127	172	220	163	213	151	137	73

values available in the parent population of possible F-values. In most cases this number is large in comparison to the 100 values obtained by random sampling.

In addition to the randomized block set-ups of tables 3 and 4 some latin squares were tried on the 500-plant uniformity data. For 50 random assignments for a 10 x 10 latin square two F's were significant at the 1% level and five at the 5% level. The mean was 1.26 and variance 0.36. For 50 random assignments for a 5 x 5 latin square one F was significant at the 1% level and seven at the 5% level. The mean was 1.60 and variance 2.07. The F's for the latin squares were calculated using interaction as the error term and not the variances due to differences between plants within primary plots. The latin squares seem to find significant differences about two or three times as often as expected.

Real variety differences ranging from $-$ 20 to $+$ 20 grams per plant and differing by 4 grams, except that the two center "varieties" differ by 8 grams, were imposed on two plot arrangements of table 4. The mean yield for table 2 is 173.4 grams, and thus the magnitudes of the superimposed real differences had a range of about 23 per cent of the general mean. The variance of these real differences was twice as large as needed for a probability of 0.8 in detecting the falsehood of the null hypothesis at the 1% level. The results are given in table 5.

TABLE 3. MEANS, VARIANCES, AND NUMBERS OF VALUES BEYOND CERTAIN LIMITS FOR 100 SAMPLE *F*-VALUES COMPARED WITH THE EXPECTED VALUES FOR THE DAVIS 1946 200-PLANT UNIFORMITY TRIAL*

Size and Description of Subplots	No. of Rep.	No. of Varieties	*F*-values for Varieties/R.E.				*F*-values for RxV./R.E.				*F*-values for Varieties/RxV.			
			Mean	Variance	No. Sig. 5%	No. Sig. 1%	Mean	Variance	No. Sig. 5%	No. Sig. 1%	Mean	Variance	No. Sig. 5%	No. Sig. 1%
1. 10 plants—5 positions down a double row	2	10	(1.01)* 1.34	(0.24) 2.01	6	0	(1.01) 1.36	(0.24) 2.06	14	1	(1.28) 1.20	(1.18) 1.96	1	0
2. 8 plants—2 positions across 4 rows	5	5	(1.01) 1.22	(0.53) 0.52	7	1	(1.00) 1.19	(0.16) 0.03	0	0	(1.14) 1.18	(0.98) 1.15	4	1
3. 20 plants—10 positions down a double row	2	5	(1.01) 1.37	(0.53) 0.56	7	0	(1.01) 1.55	(0.53) 0.55	19	0	(2.00) 1.61	(∞) 3.60	4	0
4. 20 plants—5 positions across 4 rows	2	5	(1.01) 1.66	(0.53) 0.36	13	0	(1.01) 1.70	(0.53) 0.36	15	0	(2.00) 1.33	(∞) 1.03	0	0

*The values in parentheses are values expected on the basis of the classical mathematical model.

TABLE 4. MEANS, VARIANCES, AND NUMBERS OF VALUES BEYOND CERTAIN LIMITS FOR 100 SAMPLE *F*-VALUES COMPARED WITH EXPECTED VALUES FOR THE DAVIS 1948 500-PLANT UNIFORMITY TRIAL

Size and Description of Subplots	No. of Rep.	No. of Varieties	*F*-values for Varieties/R.E.				*F*-values for RxV./R.E.				*F*-values for Varieties/RxV.			
			Mean	Variance	No. sig. 5%	No. sig. 1%	Mean	Variance	No. sig. 5%	No. sig. 1%	Mean	Variance	No. sig. 5%	No. sig. 1%
1. 10 plants—5 positions down 2 rows	5	10	(1.00)* 3.32	(0.23) 13.07	86	72	(1.00) 3.53	(0.06) 12.57	100	100	(1.06) 1.00	(0.33) 1.28	5	1
2. 10 plants—1 position across 10 rows	5	10	(1.00) 1.17	(0.23) 0.21	9	0	(1.00) 1.13	(0.06) 0.01	0	0	(1.06) 1.09	(0.33) 0.30	4	0
3. 20 plants—10 positions down two rows	5	5	(1.00) 4.55	(0.51) 10.38	72	52	(1.00) 4.92	(0.13) 0.68	100	99	(1.14) 1.14	(0.98) 2.07	3	3
4. 20 plants—2 positions across ten rows	5	5	(1.00) 1.73	(0.51) 1.16	20	10	(1.00) 1.68	(0.13) 0.07	59	4	(1.14) 1.20	(0.98) 1.19	5	2

*The values in parentheses are expected values for the classical mathematical model.

TABLE 5. MEAN, VARIANCES, AND NUMBERS OF VALUES BEYOND CERTAIN LIMITS FOR 100 SAMPLE *F*-VALUES WHEN REAL DIFFERENCES ARE IMPOSED ON THE UNIFORMITY TRIAL OF TABLE 4.

Description of Set-ups	No. of Rep.	No. of Varieties	*F*-values for Varieties/R.E.				*F*-values for RxV./R.E.				*F*-values for Varieties/RxV.			
			Mean	Variance	No. sig. 5%	No. sig. 1%	Mean	Variance	No. sig. 5%	No. sig. 1%	Mean	Variance	No. sig. 5%	No. sig. 1%
1. Set-up 1 of Table 4	5	10	5.92	5.88	98	94	(1.00) 2.88	(0.06) 0.37	97	96	2.40	3.16	42	24
2. Set-up 2 of Table 4	5	10	3.30	1.52	86	68	(1.00) 0.97	(0.06) 0.09	4	0	4.55	18.26	68	59

*The values in parentheses are expected values for the classical mathematical model.

Other sets of real differences, without end, could be imposed. The ones chosen give an uniform spread at a level that should be easily detected if we were dealing with situations adequately described by classical models.

CORRELATIONS BETWEEN THE YIELDS OF PLANTS AT DIFFERENT DISTANCES

Wiebe (9) has calculated the correlation coefficients between yields of rod-rows of wheat planted one foot apart for various distances between rows. His correlations start at about 0.8 and taper off linearly with distance being still about 0.4 at 30 feet between rows. For the strawberry trials the closest plants have a correlation of less than 0.30 which is barely significant at the 5% level. This low correlation disappears very quickly with increasing distance between plants. These results are confirmed by the data on peanuts reported by Robinson, Rigney, and Harvey (5).

DISCUSSION

Certain aspects of two uniformity trials based on individual plant yields are presented and compared. Many of the random sampling results conform to the current textbook procedures of treating yield trials as far as saying differences exist when in fact they do not exist. The 200-plant trial shows much better conformity in some respects than does the 500-plant trial.

One result that is very clearly indicated is that much better results are obtained if the primary plots are taken across rows instead of down rows. This positioning of plots is confirmed by Sharpe and Blackmon (6) with pecans and Taylor (8) with strawberries in England.

Table 5 indicates that real differences may not be quite as detectable as is usually indicated. This is possibly due to the nonrandomness of soil and plant variations.

The correlation between near plants is so low as to be practically zero.

SUMMARY

Two uniformity yield trials of 200 individual plant yields and 500 individual plant yields are presented. The 500-plant trial was somewhat more variable as measured by the ratio of standard deviation to the mean.

F-values are given for certain randomized block designs with random assignment of "varieties". These *F*-values indicate that the trials are different and that some plot arrangements are better than others. In

particular primary plots running across rows are much better than primary plots running down rows.

F-values for latin square design indicate a possible tendency to find differences when they do not exist.

Correlations between plants close together are low.

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ANALYSIS OF A TRIPLE RECTANGULAR LATTICE DESIGN

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Harshbarger (1946, 1947, 1949) developed a set of incomplete block designs in which the number of varieties (or treatments) is the product of two consecutive integers and the number of replications of each variety is either 2 or 3 and their multiples. He called them *simple* and *triple rectangular lattices* respectively.

K. R. Nair (1951) has shown that a Triple Rectangular Lattice when $v = 3 \times 4$ is a p.b.i.b. (partially balanced incomplete block) design with m equal to 3. In the June 1952 issue of Biometrics he has given the analysis of Simple Square and Rectangular Lattices. The object of the present note is to give a numerical illustration of the Analysis of Triple Rectangular Lattice Design following the methods of K. R. Nair. For the purpose the data for illustration have been taken from p. 295 of Experimental Designs by Cochran and Cox. This example is specially interesting as the Intra Block error is zero.

The parameters of the design are:

$$v = 12, \quad k = 3, \quad r = 3, \quad b = 12,$$

$$\lambda_1 = 1, \quad \lambda_2 = 0, \quad \lambda_3 = 0,$$

$$n_1 = 6, \quad n_2 = 3, \quad n_3 = 2,$$

$$p^1_{ik} = \begin{Bmatrix} 2 & 2 & 1 \\ 2 & 0 & 1 \\ 1 & 1 & 0 \end{Bmatrix}, \quad p^2_{ik} = \begin{Bmatrix} 4 & 0 & 2 \\ 0 & 2 & 0 \\ 2 & 0 & 0 \end{Bmatrix}, \quad p^3_{ik} = \begin{Bmatrix} 3 & 3 & 0 \\ 3 & 0 & 0 \\ 0 & 0 & 1 \end{Bmatrix}.$$

The matrix

$$\begin{Bmatrix} A_{13} & B_{13} & C_{13} \\ A_{23} & B_{23} & C_{23} \\ A_{33} & B_{33} & C_{33} \end{Bmatrix} \equiv \begin{Bmatrix} 6 & -1 & 0 \\ -3 & 7 & -1 \\ 3 & 1 & 9 \end{Bmatrix}$$

The determinant of the matrix

$$\Delta = 360.$$

The twelve varieties may be represented schematically.

X	1 124	2 132	3 143
4 213	X	5 234	6 241
7 314	8 321	X	9 342
10 412	11 423	12 431	X

This scheme enables one to write down quickly the first, second and third associates of any given variety.

The total S.S. and the Block S.S. (ignoring varieties) are calculated in the usual manner. The varieties S.S. (eliminating blocks), is calculated from the table given below.

TABLE 1. INTRA BLOCK ESTIMATES OF VARIETAL EFFECTS

Variety No.	(α)	(β)	(γ)	(δ_1)	(ϵ_1)	(δ_2)	(ϵ_2)	(δ_3)	(ϵ_3)	(η)	(ξ)
1.	55	123	42	2,3,8,11,5,7	−8	4,9,12	−36	6,10	2	2580	18.67
2.	34	94	8	1,3,5,12,9,10	−54	6,7,11	34	4,8	12	60	11.67
3.	18	87	−33	1,2,6,9,4,11	43	5,8,10	−15	7,12	5	−1740	6.67
4.	6	49	−31	5,6,7,10,3,11	−57	1,9,12	37	2,8	51	−2460	4.67
5.	20	89	−29	4,6,2,12,1,7	55	3,8,10	−19	9,11	−7	−1380	7.67
6.	46	107	31	4,5,3,9,8,12	−55	2,7,11	11	1,10	13	1500	15.67
7.	47	129	12	8,9,4,10,1,5	−2	2,6,11	30	3,12	−40	780	13.67
8.	62	143	43	7,9,1,11,6,12	71	3,5,10	−91	2,4	−23	3300	20.67
9.	48	142	2	7,8,3,6,2,10	32	1,4,12	4	5,11	−38	420	12.67
10.	22	95	−29	11,12,4,7,2,9	−25	3,5,8	−19	1,6	73	−2100	5.67
11.	25	84	−9	10,12,1,8,3,4	−15	2,6,7	51	5,9	−27	−660	9.67
12.	31	100	−7	10,11,2,5,6,8	15	1,4,9	13	3,7	−21	−300	10.67
Total	414	1242	0		0		0		0	0	

(α) = Total yield of the variety.
(β) = Total of the Blocks in which the variety occurs.
$(\gamma) = k.\ (\alpha) - (\beta)$
(δ_1) = First associates of the variety.
(ϵ_1) = Sum of (γ) for varieties in (δ_1)
(δ_2) = Second associates of the variety.
(ϵ_2) = Sum of (γ) for varieties in (δ_2)
(δ_3) = Third associates of the variety.
(ϵ_3) = Sum of (γ) for varieties in (δ_3)
$(\eta) = [B_{23}C_{33} - B_{33}C_{23}]\ (\gamma) - B_{13}\ [C_{33}\ (\epsilon_1) - C_{23}\ (\epsilon_2)] + C_{13}\ [B_{33}\ (\epsilon_1) - B_{23}\ (\epsilon_2)]$
$(\xi) = (\eta)/\Delta$ + Grand Mean = Adjusted varietal mean

$$\text{S.S. due to varieties (eliminating blocks)} = \frac{\sum(\gamma)(\eta)}{k \cdot \Delta} = \frac{550080}{3 \times 360} = 509.33$$

TABLE 2. ANALYSIS OF VARIANCE

Source	D.F.	S.S.	S.S.	Source
Blocks (ignoring varieties)	11	761.67	204.00	Blocks (eliminating varieties)
Varieties (eliminating blocks)	11	509.33	1067.00	Varieties (ignoring blocks)
Intra-Block error	13	0.00	0.00	Intra-Block error.
Total	35	1271.00	1271.00	

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QUERIES

GEORGE W. SNEDECOR, *Editor*

103 QUERY: Comment on Query No. 96, Vol. 8, December 1952, page 383. By C. B. Williams, Department of Entomology, Rothamsted Experimental Station.

"I find myself in disagreement with the comments of Professor Snedecor in the problem of a 'missing-plot' in an experiment on the attraction of insects to bait traps, as discussed by him in Biometrics.

"The submittor of the problem had made 12 repetitions of 4 different baits as attractants for fruit flies, the number of insects caught per trap per repetition varying from 3 to 75. In one repetition one bait was missing, and when the usual formula was employed to find a replacement for this the number came to -6.64!! The questioner asked 'How do you explain this? Have we made a mistake?'

"Professor Snedecor's comment was that no error had been made in the calculation, and that the value obtained 'is not intended as an estimate of the missing datum. It is merely a number to be put in the empty space so that you can conveniently extract the remaining information from the experiment'.

"This may be good mathematics but it is biologically meaningless. The repetition in question had very low values for the other three baits (6, 16 and 23), and the missing bait was on an average the poorest of the four, so that had a proper figure been obtained experimentally it would probably have been between 0 and 10. But to suggest that any increased accuracy (which is surely the object of mathematical reasoning) is obtained by putting a *negative* value, cannot possibly be upheld.

"When an absurd result is obtained by mathematical reasoning either the mathematics is wrong or else it is based on false premises. In the case under consideration the fundamental assumption has been made by the experimenter—and accepted (with one qualification) by Professor Snedecor—that the differences between baits within a single repetition, and the differences between repetitions of a single bait, can be soundly based on an arithmetic scale. In other words it is assumed that a particular bait tends to attract a fixed number n more (or less) insects than another bait irrespective of the general level of catch.

"Much experience in problems of comparing changes in insect numbers, particularly as caught in traps, has however convinced me that differences between catches are usually in similar ratio or proportion—a particular trap tends to catch n per cent more or less than another. Once this is recognised it follows that such data can only be soundly compared on a geometric, or logarithmic scale.

"Details of this work will be found in the following publications:

Philos. Trans. B. 226, 361(1936); Ann. Appl. Biol. 24(2):404 (1937); Trans. R. ent. Soc. Lond. 90:234–239 (1940) and Bull. ent. Res. 42(3):513–517 (1951). The last reference deals specifically with the problem of comparison of traps with different attractants.

"To apply the method to the problem under discussion the number of insects caught in each bait repetition must be converted to a logarithm, and if this is given to two decimal places an accuracy of about 2% is obtained. The original table as quoted by Snedecor then becomes as shown below—

Repetitions	BAITS				
	A	*B*	*C*	*D*	Total
1	0.90	1.88	1.61	1.67	6.06
2	0.70	1.72	1.48	1.46	5.36
3	0.90	1.76	1.58	1.40	5.64
4	0.48	1.28	1.30	1.00	4.06
5	1.23	1.61	1.54	1.90	6.28
6	1.18	1.53	1.45	1.11	5.27
7	0.95	1.69	1.38	1.08	5.10
8	1.28	1.75	1.36	1.26	5.65
9	——	1.20	0.78	1.36	(3.34)
10	1.30	1.52	1.42	1.23	5.47
11	0.85	1.40	1.18	1.08	4.51
12	1.32	1.82	1.59	1.32	6.05
Total	(11.09)	19.16	16.67	15.87	(63.45)

"The missing value is given by

$$X = \frac{4(11.09) + 12(3.34) - 63.45}{33} = 0.66$$

"The anti-log. of this is 4.6 which is the estimated number of insects likely to have been caught at bait *A* in the 9th repetition. This estimate is both mathematically sound and biologically reasonable.

"To continue the analysis it follows that, after incorporating the missing term, the mean log. catches per repetition with the four baits are:—

0.98 : 1.60 : 1.39 and 1.32

so that the geometric mean catches are:—

9.5; 39.8; 24.6 and 20.9

The corresponding arithmetic means on the original analysis (including the missing term) are:

12.5; 43.7; 27.1 and 25.5

These two sets of numbers appear at first sight to be somewhat similar, but their interpretation is very different. The geometric technique implies that if trap A gets x insects, then trap B will get $4.2x$, trap C $2.6x$ and trap D $2.2x$. But the arithmetic method implies that if trap A gets x insects then trap B will get $x + 31$, trap C will get $x + 15$, and trap D $x + 13$."

ANSWER: On one point I heartily agree with Dr. Williams: the occurrence of any unusual feature, such as the negative value of the missing plot, should cause the investigator to re-examine both his theory and his experimental procedure. On other points my views are less extreme.

As intimated in the original answer, there are logically satisfying reasons for making a transformation. The counts of insects may follow Poisson distributions in which the variances change with the means. The unequal variances vitiate both the estimates and the tests of hypotheses. An appropriate transformation alleviates the difficulty. But no conclusion would be changed in the experiment under discussion. In my experience this situation has occurred so often that I tend to be a bit lax about transformations in this type of data unless close decisions are anticipated.

As for a negative "missing value", I do not consider it absurd nor as necessarily indicating anything wrong with either the model or the data. It may arise merely from the incidents of sampling. If the model is appropriate and if the experiment is successfully performed, the use of the missing plot technique, with well known corrections for bias, leads to unbiased estimates of the treatment means and to unbiased tests, the sign of the missing value being irrelevant. As we have often emphasized, these estimates and tests can be arrived at without the calculation of a missing value; it is merely a device for making available the more familiar forms of calculation. True, a negative value would not ordinarily have biological meaning but, in my experience, there is nothing to be learned from assigning a meaning to it; usually it is only the treatment means and a measure of experimental error that are needed and these may be estimated correctly irrespective of the sign of the missing value.

It happens then that in this particular experiment the tests of hypotheses lead to the same conclusions with or without the transformations. But, because of the unequal weightings associated with the counts, the treatment means are doubtless better estimated from the transformed data.

ABSTRACTS

Indian Region, New Delhi, February 26, 1953

232 D. J. FINNEY. (Indian Council of Agricultural Research, New Delhi.) **The Choice of Levels.**

In this paper were outlined considerations involved in the choice of numerical levels of a quantitative factor to be used in an experiment. The choice must depend upon the purpose of the experiment and the extent of existing knowledge. The principles were illustrated by reference to the choice of doses for a slope-ratio biological assay and the choice of levels of fertilizer to be used in experiments for determining the economic optimal dressing.

233 K. R. NAIR. (Forest Research Institute, Dehra Dun.) **Some Unsolved Problems in Experimental Designs.**

The paper points out an unsolved problem in the construction of optimum confounded designs of an $s_1 \times s_2$ factorial experiment in blocks of s_2 plots where s_1 and s_2 are mutually prime and $s_1 < s_2$. It also cites general problems yet to be solved in extending the results to the general asymmetrical factorial experiment involving more than two factors and expresses the need for fundamental investigations into incomplete factorial experiments, sequential experimentation, etc.

234 K. KISHEN. (Uttar Pradesh Statistician, Department of Agriculture, Lucknow.) **Some Unsolved Problems in Experimental Designs.**

Dr. K. Kishen gave an account of his investigations into the problem of constructing generalized asymmetrical factorial designs. He gave two general solutions, but said that the class of designs he had investigated were not optimum designs and that there was need for investigating such designs.

235 A. ANANTHAPADMANABHA RAU. (Mysore State Agricultural Department, Bangalore.) **Some Difficulties in Experimentation with Coffee—A Plantation Crop.**

A plantation crop like coffee offers difficulties additional to those offered by annual crops to a Field Experimenter. High variability in

yields between plants in the same year, year to year variability in yields of the same plant, in addition to the correlation between yields of successive years and the cyclic trends, are mainly responsible for field experiments with coffee being, in general, unsuccessful in giving significant results. Exhaustive study of yield data on coffee in uniformity trial and manurial trials has pointed out the necessity for a new approach to this problem. Inconsistent results are obtained by the application of usual methods of analysis of variance and orthogonal polynomials—frequently applied to data with trends—as these do not satisfactorily represent the trends present in coffee yield data. Application of other methods has given some significant results regarding the variability and the manurial effects. These are discussed.

236 V. G. PANSE. (Indian Council of Agricultural Research, New Delhi.) **Long Term Experiments.**

The paper outlined the general principles governing the design of long term experiments in agriculture and described a rotational cum manurial trial in progress at the Institute of Plant Industry, Indore, since 1947. The experiment involves comparison of two crop rotations and the response of the rotation crops to three types of nitrogen, four frequencies of its application and application of phosphate. The experiment is laid out in two replications, each consisting of seven crop plots, three for one rotation and four for the other. In each crop-plot there are 24 main plots for nitrogen and frequency of application treatments, and each main plot is sub-divided into three sub-plots for phosphate treatment. The experiment thus includes a total of 1008 sub-plots and is planned to run for 12 years in the first instance. The results obtained so far showed that cotton following groundnut in the second rotation gives a substantially higher yield than when it follows a millet in the first rotation and that the response of crops to nitrogen from ammonium sulphate and oilcake is distinctly superior to that from farmyard manure.

237 UTTAM CHAND. (Indian Council of Agricultural Research, New Delhi.) **Experiments on Tree Crops.**

The paper presents a brief review of some of the experiments which are being done in India to determine the manurial requirements of coconut, citrus, cardamom and pepper. In drawing up manurial and cultural experimental programmes on tree crops flexibility of any recommended design with a view to introduce other factors in the future which are orthogonal to the first set should receive particular attention. With this end in view asymmetrical designs should be avoided as far as possible.

The paper makes a plea for more intensive agricultural research to determine the rate at which manurial doses should be increased with advancing age of the trees.

238 V. N. AMBLE. (Indian Council of Agricultural Research, New Delhi.) **Animal Experiments.**

The paper described the salient features of switchback and switchover trials which are of use in animal experiments and indicated how the extension of the switchback trial to an even number of periods beyond two would permit the examination of the presence of the carryover effect even in cases where only two sets of animals are allotted to the two switchback schedules. Where, however, carryover effect is initially suspected and estimation of purely direct effect is desired, two additional sets of animals put on the two treatments continuously would be necessary.